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Development and *In Vitro* Evaluation of Ceramic Nanoparticles of Piroxicam

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***SUMMARY*. The objective of the present study was to prepare ceramic nanoparticles of a poorly aqueous soluble drug (piroxicam) to explore the relationship between particle size and dissolution profile. Ceramic nanoparticles were prepared by self-assembling of hydroxyapatite using colloidal precipitation technique, with the aid of refluxing. Then the nanoparticles were coated with trehalose (polyhydroxyl oligomer) and subsequently piroxicam was allowed to adsorb. These ceramic nanoparticles were characterized for the shape, size, size distribution, yield, drug loading and release profile. The SEM analysis indicated spherical particles with a particle size median of 238 nm. The percent yield of ceramic nanoparticles was 66.7 %. The dissolution profile of piroxicam aquasomes was obtained in 0.1 mol/L hydrochloric acid solution. The release of piroxicam from ceramic nanoparticles was linear and exhibited zero order kinetics. Studies indicated that the piroxicam ceramic nanoparticle formulations elicited release of piroxicam in 1 h 15 min. This result also indicated specific interaction of piroxicam and trehalose. In the absence of sugar adsorption, the piroxicam release was to the tune of 90% in 60 min., which can be usefully exploited for the immediate action.**

INTRODUCTION

Development of solid dosage forms for water-insoluble drugs has been a major challenge for pharmaceutical scientists for decades. The drug efficacy can be severely limited by poor aqueous solubility because the driving force is concentration gradient of drug in solution, for absorption across biological membranes. The consequences include low bioavailability, large inter- and intra- subject variation and fluctuations in blood drug concentrations between fed and fasted conditions. A number of developments have been introduced with an intention of reducing the drug toxicity and dosage requirement, enhancing solubility, bioavailability, cellular targeting. Prodrugs, macromolecules and liposomes have served to attain the above intention. However, all these are prone to have biophysical constraints. The destructive interactions between the carrier and the drug are often inevitable and these always bring limitations to the drug delivery system. In such a circumstance, the ceramic nanoparticles are worth promising carriers, which prevents denaturing of drug and imparts hydrophilicity to the formulation [1]. Over the years, approaches for enhancing the drug delivery have made considerable advances. One that significantly contributed to the progress is the submicron or nano delivery systems. They were successfully used in the delivery of anticancer drugs [2], controlled release of plasmid DNA [3], brain drug delivery [4], and nasal drug delivery [5].

Nanoparticulate carrier systems constitute one of the self-assembling approaches for the delivery of bio-active agents [1]. Molecular self-as-

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sembly is the spontaneous association of molecules into stable, structurally well-defined aggregates, joined by non-covalent bonds [6,7]. Self-assembly generates structures which occupy the condition of thermodynamic minima. Ceramic nanoparticles (aquasomes), first developed by Nir Kossovsky, are a new drug delivery system comprised of surface modified nanocrystalline ceramic carbohydrate composites to which drug moieties or biochemically active molecules are adsorbed with or without modification [8-11]. The large active surface can be used for efficient loading. Solid particles dispersed in aqueous environment exhibit physical properties of colloids [7]. Carbohydrate film imparts water-like properties that help to protect and preserve the fragile biological molecules even in the dry state [1,7]. Natural sugars act as dehydroprotectants because of the hydroxyl groups present and preserve the aqueous structure of biological molecules on dehydration. The decrease in particle size and enhanced surface area contact are the approaches used to improvise the drug solubility, though polyhydroxy film imparts hydrophilicity.

Ceramic nanoparticles were studied for delivery of antigen, superior surface inimitability by preserving structural integrity of protein and thus better immunological response was observed [8]. The study on haemoglobin ceramic nanocarriers established the superiority of oxygen carrying capacity and suggested as an artificial blood substitute [9]. Insulin ceramic nanoparticles provided conformational stabilization, prolonged activity and protection of the spatial properties of the insulin and exhibited better therapeutic effect than insulin solution [10]. Prolonged activity was observed without denaturation during storage of enzyme, serratiopeptidase in the form of gel encapsulated nanocores [11]. Most of the studies were conducted on biological molecules. All these studies were conducted for parental delivery. The present work is to use the technology for enhancing the solubility of poorly soluble drugs and also intended for oral use.

The drug chosen for the investigation is piroxicam, a non steroidal anti-inflammatory drug. It is a poor water soluble drug, belonging to class II of Biopharmaceutical Classification System (*i.e.* low solubility and high permeability). The dose of piroxicam is 10 to 20 mg daily and in acute conditions it can be administered up to 40 mg daily. The solubility is very low, but well absorbed after oral administration. Peak blood levels are reached between three and five h [12,13], which are considered as not acceptable, if rapid release and instantaneous onset of action is desirable. There is a need to improve piroxicam dissolution profile and in turn its bioavailability. Therefore, formulation of piroxicam was attempted using ceramic nanoparticle system for oral drug delivery. Carbohydrate stabilized ceramic cores were coated with piroxicam. These drug loaded ceramic nanoparticles were evaluated for dissolution characteristics.

MATERIALS AND METHODS

Materials

Piroxicam was obtained from Strides Arcolab Limited, Bangalore, as a gift sample. Trehalose was obtained from Loba Chemie Pvt. Ltd., Mumbai. All other chemicals were of analytical grade and used as received.

Preparation of ceramic nanoparticles

By using the principle of self assembly, ceramic nanoparticles are prepared by a three-step method that involves production of ceramic cores (calcium phosphate), adsorption of sugar (trehalose) on ceramic core, and immobilization/ adsorption of piroxicam on sugar-coated ceramic.

Preparation of core

Ceramic core was prepared by coprecipitation technique with magnetic stirring under reflux conditions [9]. Diammonium hydrogen phosphate solution (0.19 N) was added drop-wise under continuous stirring to calcium nitrate solution (0.32 M), which was maintained at 75 °C, in a three-necked flask bearing one charge funnel, a thermometer, and a reflux condenser. During the addition, the pH of calcium nitrate was maintained between 8-10 using concentrated aqueous ammonia solution. The pH was tested with litmus paper. The mixture was then stirred for 6 days. The precipitate was filtered, washed thoroughly with double distilled water, and air-dried overnight at 100 °C. The powder was then sintered by heating to 800-900 °C in an electric furnace. The reaction can be given as follows.

$$3\ (NH_4)_2HPO_4 + 3\ Ca(NO_3)_2 \rightarrow Ca_3(PO_4)_2 + 6NH_4NO_3 + H_3PO_4 \quad (75\ °C\ /\ pH\ 8\text{-}10)$$

In order to study the effect of pH and time on the hydroxyl-apatite core formation, particles were also prepared without pH control and also with 1 day of stirring.

Adsorption of sugar on ceramic core

The optimized ceramic cores were coated with trehalose by adsorption method [9,10]. The hydroxy-apatite cores were weighed (approximately 500 mg) and placed into a cleaned and dried iodine flask. Trehalose solution (5 mL, 5 mg/mL) was transferred into the flask and volume was made up to 50 mL with water. The flask was stoppered and shaken vigorously for about 20 min and then suspended in a trough containing water at room temperature about 1 hour with intermittent shaking. The suspension was centrifuged at 2000 rpm for 5 min. The sugar-coated ceramics were later dried. The extent of sugar coating was measured using anthrone method.

Quantification of trehalose coating on core using anthrone reagent

Trehalose coating on ceramic core was quantified using anthrone method [14,15]. The principle involved is as follows: carbohydrates are first hydrolyzed into simple sugars which in hot acidic medium are converted to hydroxyl methyl furfural, which reacts with anthrone and forms a green colored product. A calibration curve (in the range of 0-20 µg/mL) was prepared from a standard stock solution of trehalose (100 µg/mL). For this, aliquots of samples (0, 0.2, 0.4, 0.8, and 1 mL) were transferred into boiling tubes and made up to 1 mL using distilled water; anthrone reagent (5.5 mL) was added, heated in a boiling water bath (10 min, 100 °C) and cooled rapidly. A greenish solution was obtained whose absorbance was recorded (λ_{max} of 625 nm) using a UV-visible spectrophotometer (UV-1700, Shimadzu Corporation, Japan). Sugar coated ceramic core (50 mg) was accurately weighed and dissolved in distilled water (5 mL). From this stock, solution (2 mL) was taken and anthrone reagent (5.5 mL) was added and boiled (10 min, 100 °C). The solution was cooled rapidly and the absorbance was estimated at 625 nm.

Adsorption of piroxicam on the trehalose-coated core

Piroxicam solutions (1.0 to 2.5% w/v in acetone) were added to iodine flasks containing weighed amount of sugar-coated ceramics. The flasks were stoppered and shaken at 130 rpm, at 12.5 °C (also carried out at 6-8 °C) for 24 h (optimized conditions) using orbital shaker incubator (Kemi, Kerala), then suspended in a trough containing water at room temperature and left for about 1 hour with intermittent shaking to produce three-layered [16] drug loaded ceramic nanoparticles. The suspension was centrifuged at 15,000 rpm for 5 min in a cooling centrifuge (Remi, Mumbai) at 12.5 °C and ceramic nanoparticles separated and dried [10].

Plain piroxicam nanoparticles *i.e.* loading of drug directly on ceramic core without sugar layer, were prepared in a similar manner by eliminating the sugar adsorption step. These nanoparticles were used for further comparative study with ceramic nanoparticles.

Evaluation Of Ceramic Nanoparticles

The piroxicam ceramic nanoparticles were characterized for particle size, size distribution analysis, and piroxicam payload.

Particle size and size distribution analysis

The average size and size distribution of ceramic nanoparticles were determined by scanning electron microscope (Hitachi S-3000N, Ireland) in which the samples were mounted rigidly on the surface of a bronze-specimen holder (called a specimen stub) using a double-sided adhesive tape and coated with an ultra-thin coating of electrically-conducting material, gold, deposited on the sample either by low vacuum sputter coating or by high vacuum evaporation with gold and observed under suitable magnification [7,10,17].

Piroxicam payload

The piroxicam payload in the ceramic nanoparticles was determined by the following procedure [9,10]. Ten mg of the piroxicam ceramic nanoparticles was dissolved suitably in 10 mL methanol. This dispersion was completely dissolved in 100 mL of 0.1 mol/L hydrochloric acid solution. Absorbance of this solution was measured spectrophotometrically at 334 nm (λ_{max}) [13]. For the determination of piroxicam, calibration curve was developed and Beer Lambert's law was obeyed in the range of 2-10 µg/mL in 0.1 mol/L hydrochloric acid solution. Percent payload was calculated using the following formula: *% Pay Load = (Amount of drug in aquasomes / Amount of aquasomes) × 100.*

In vitro release studies of ceramic nanoparticles of piroxicam

In vitro release studies were carried out in 0.1 mol/L hydrochloric acid solution. Piroxicam-loaded ceramic nanoparticles were weighed (equivalent to 10 mg of piroxicam) and packed

into capsules (size 0). The intension is to evaluate the ceramic nano particles as such (as modified API), rather than formulating into capsules. Dissolution was performed as reported in USP/NF using USP type I dissolution apparatus under pharmacopoeial conditions (50 rpm, 37 ± 0.5 °C). Samples were withdrawn at predetermined time intervals and replaced by fresh medium. The samples were analyzed for piroxicam content [18]. Simultaneously, dissolution studies of capsules containing commercial piroxicam (10 mg) and piroxicam loaded directly on ceramic nanocore *i.e.,* piroxicam nanoparticles (equivalent to 10 mg piroxicam) were carried out for the comparison [10,13].

To study the release kinetics, data obtained from *in vitro* drug release studies were plotted in various kinetics models (zero order, % Drug release = k. Time and first order, Log (fraction unreleased) = (k/2.303) x time) to understand the linear relationship, *i.e.,* kinetic principles. The data were processed for regression analysis using Ms Excel statistical functions. To study the release mechanisms, the data of *in vitro* drug release was verified using Higuchi's model (M = $kt^{1/2}$, where M = percentage of drug released, t = time, k = proportionality constant) and Hixson Crowell Cube root law ($M_0^{1/3}$ - $M_t^{1/3}$ = kt, where M_0 = mass of the drug particles initially, t = 0, M_t = mass of the drug particles at time, t, k = proportionality constant, t = time) models.

RESULTS

Ceramic nanoparticles of piroxicam were developed with a view to improve the dissolution of poorly soluble drug on oral administration.

Preparation of core

Calcium phosphate core was prepared using coprecipitation technique under reflux conditions reported by Patil *et al.* [9]. Initially, pH was not maintained. The results were reported in Table 1. It was observed that the coprecipitation method with uncontrolled pH produced crystalline ceramic core in micrometer size. When pH (8-10) was controlled, round particles were produced in the size range of nanometer to micrometer. Further, on sintering at 800 to 900 °C, spherical particles in nanometer size were produced. At pH 8-10, core was prepared at two time periods of stirring, 1 day and 6 days (4-6 days). Observations indicated that the average particle size of the ceramic nanoparticles decreased with increasing time of stirring (500-1000 nm to 100-200 nm). Thus the pH and stirring time were finalized as 8-10 and 6 days, respectively.

Sugar coating on the ceramic core

The ceramic nanoparticles prepared by above method were coated with trehalose using adsorption technique. The extent of sugar coating was measured using anthrone method and found to be 87.56 ± 0.864 µg/ 100 mg of core.

Loading of piroxicam on sugar coated core

Piroxicam was loaded onto trehalose coated core using adsorption technique. Percent payload was increased with increasing drug concentration for the given conditions: temperature (6-8 °C), contact period (1.5 h) and intensity of shaking (130 rpm). The results indicated that the percent payload of ceramic nanoparticles was 0.127 ± 0.01 and 1.367 ± 0.25 at piroxicam concentrations of 0.1% w/v and 1.0% w/v, respectively. Such a low pay load is not preferable. Hence, the contact period was increased to 6 h (6-8 °C; 130 rpm). Then the payload was enhanced to 1.467 ± 0.12% at piroxicam concentration of 1.0% w/v. There was a slight increase

Parameters	Conditions	Size range	Nature of particles	% Yield
pH	uncontrolled	1.0-10 µm	elongated	37
	8 to 10	≤ 1 µm	elongated to spherical	36
		100-200 nm (with sintering)	spherical	60
Time of slurry (pH 8-10)	1 day	≤ 10 µm	elongated	33
		500- 1000 nm (with sintering)	elongated	33
	4-6 days	250 - 1000 nm	elongated to spherical	61
		100 -200 nm (with sintering)	spherical	60

Table 1. Effect of pH and time on the formation of hydroxy-apatite core.

Piroxicam concentration (% w/v) for incubation	Payload of ceramic nanoparticles, (%) *A.M. ± S.D.		
	Ceramic nanoparticles		Nanoparticles without sugar adsorption
	6-8 °C	12.5 °C	12.5 °C
1	1.60 ± 0.26	1.74 ± 0.36	0.59 ± 0.45
1.2	2.07 ± 0.21	3.24 ± 0.23	-
1.3	2.23 ± 0.21	3.95 ± 0.18	-
1.4	2.70 ± 0.26	4.65 ± 0.59	-
1.5	26.06 ± 41.51	6.47 ± 0.44	2.56 ± 0.61
2	66.66 ± 5.25	72.04 ± 25.24	-
2.5	150	-	-

Table 2. Percent payload of piroxicam at different drug concentrations at different temperatures for a contact period of 24 hr. *Each value represents the mean of 3 determinations. Note: Percent payload was calculated using equation, % payload= amount of drug in aquasome/amount of aquasome x 100.

in drug payload when the contact time was increased. Keeping other parameters constant, incubation time was increased up to 24 h, the piroxicam concentration was varied (1.0, 1.2, 1.3, 1.4, 1.5, 2.3, and 2.5% w/v in acetone) to find the optimum drug concentration (Table 2). Studies indicated that the percent payload increased with increasing drug concentration. Further incubation was attempted at higher temperature, 12.5 °C (Table 2). Considerable increase in the payload (6.471%) was achieved at 12.5 °C (for 1.5 % w/v piroxicam concentration).

Non-sugar adsorbed nanocores were also loaded with piroxicam. At a concentration of 1% w/v and 1.5 % w/v, plain nanocores show a payload of 0.598 and 2.565% w/w respectively, which was quite low compared to sugar adsorbed ceramic nanoparticles (1.738 and 6.471% w/w payload, respectively). Hence, sugars play a vital role in the enhancement of piroxicam loading (Table 2, serial no. 5). After drying, the drug-loaded ceramic nanoparticles was free-flowing. The ceramic nanoparticles were collected and weighed. Percentage yield of the nanoparticles was found to have a good yield (66.7%).

Particle size and size distribution analysis

The SEM micrographs (Fig. 1) revealed that piroxicam ceramic nanoparticles are spherical with a smooth surface. These exhibited the size distribution in the range of 60- 300 nm. SEM was attempted at the three stages of preparation, *i.e.,* core formation, sugar loading on the core and finally the piroxicam loaded ceramic nanoparticles. The particle size distributions were also graphically expressed in Fig. 2. The size range was increased after each step, as indicated in medians of 173.18, 196.44 and 238.4 nm respectively. Particle size of drug loaded formulation was within the desired size range of 60-300 nm, while commercial piroxicam showed

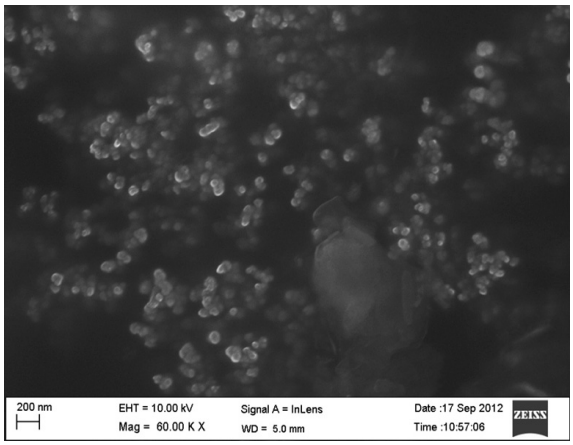

Figure 1. SEM micrograph of proxicam ceramic nanoparticles.

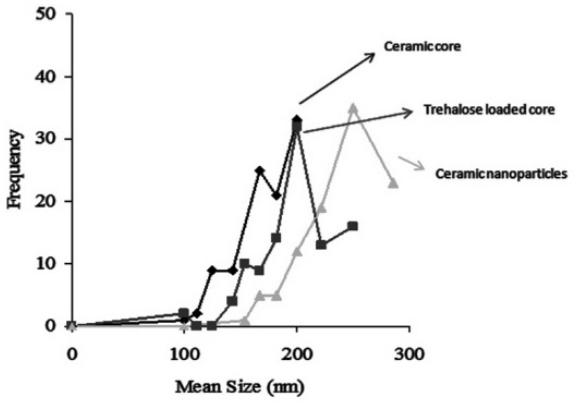

Figure 2. Size distribution analysis of particles during the preparation of piroxicam ceramic nanoparticles. Commercial sample has 4545 nm.

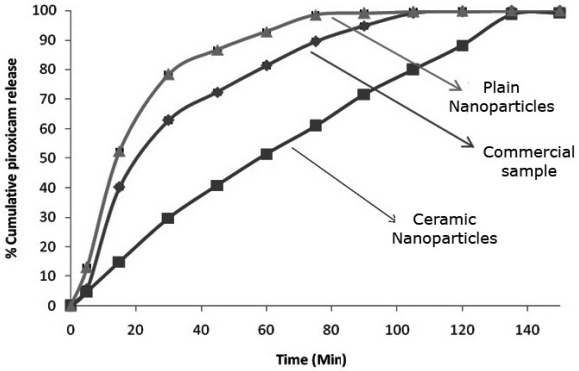

Figure 3. *In vitro* drug release profiles from various piroxicam formulations.

a particle size of 4.545 µm (Trinocular microscope, Magnus, India). Thus, the piroxicam formulation was in nanosize for the expected enhancement of its aqueous solubility.

In vitro release kinetics of ceramic nanoparticles of piroxicam

The cumulative piroxicam release was obtained from ceramic nanoparticles, plain nanoparticles and commercial piroxicam (Fig. 3). Piroxicam release was observed to be about 90% in 60 min from plain piroxicam nanoparticles, whereas the release was 75 mins from commercial piroxicam and 130 mins for ceramic nanoparticles. The release of drug from direct ceramic cores was better than that of pure piroxicam at all times of study. This enhancement in the dissolution profile could be credited to nano-sizing of the particles.

DISCUSSION

The first step is the preparation of ceramic core. Ceramic is a material of choice, as it is structurally known to be most regular. Therefore surface modification will have only a limited effect on the nature of the atoms and thus the bulk properties are preserved. The surfaces exhibit high level of surface energy that will favor the binding of polyhydroxy oligomeric surface film [7]. In this co-precipitation technique under reflux conditions, a change in size and shape was observed when pH conditions were altered. When pH was maintained at 8-10, spherical particles in nanometer range were obtained upon sintering.

The change in size and shape can be attributed to the solubility of calcium phosphate. Under uncontrolled pH, the dispersion reaches acidic levels, because of the release of nitric acid. Calcium phosphate is soluble in dilute nitric acid, which gave less yield and non spherical shape. In alkaline pH, the insoluble core tends to take up a spherical shape under shear stress. When the stirring time was increased from 1 day to 6 days, average particle size was decreased. The reason could be that the spherical particles were subjected to more shear stress (mixing) for a prolonged period. The increased time of contact is necessary to complete the reaction and to obtain high yield of core (60.8%) (Table 1).

The second step in the preparation is coating of ceramic core with trehalose. Trehalose was taken due to its good binding characteristics onto the hydroxy- apatite core [9]. Trehalose arranges itself in a manner in which lowest energy of adsorption is achieved. Good amount of trehalose adsorption was achieved (87%). A corresponding increase in size was achieved. These were supported by SEM.

The third step involved is piroxicam loading, which was achieved by adsorption method. Optimum conditions were selected to get reproducible results. Drug payload was increased with increase in piroxicam concentration. At and above 1.5 w/v % of piroxicam, there was a rapid increase in payload. This was assumed to be due to crystallization of drug in the solution, due to saturation. Thus unusual drug payload was obtained. The piroxicam loading need to be due to surface adsorption on the sugar coated cores, rather than crystallization. Further the incubation temperature was increased to 12.5 °C (Table 2). Temperature could not be further elevated as it leads to drastic increase in the rate of vaporization of acetone. Thus, the operating temperature had been optimized at 12.5 °C and drug concentration at 1.5 %.

In vitro dissolution studies were indicated that the trehalose loaded piroxicam ceramic nanoparticles released the drug in a controlled manner. The dissolution of piroxicam from commercial sample followed first order release (Table 3) and justified. In fact piroxicam powder dissolution was expected and Hixson-Crowell cube root law must be applicable. But the mechanism was found to be diffusion controlled. This anomalous behavior was attributed to solubility related phenomena. The release of piroxicam from ceramic nanoparticles predominantly followed zero order kinetics and diffusion controlled.

Formulation	Equations and R^2			
	Zero	First	Higuchi	Hixson-Crowell Cube Root
Commercial Piroxicam	y = 1.7355x+5.7121 R^2 = 0.8794	y =-0.0134x+1.9972 R^2 = 0.9345	y =12.853x- 10.7262 R^2 = 0.9037	y =-0.0779x + 3.2326 R^2 = 0.7281
1.5% ceramic nanoparticles	y = 1.0515x+0.6585 R^2 = 0.9972	y = -0.0061x+ 2.008 R^2 = 0.9976	y =7.4207x- 7.7176 R^2 = 0.9304	y = -0.0636x+ 3.4692 R^2 = 0.7856
1.5% Plain nanoparticle	y = 1.9322x+13.5561 R^2 = 0.8939	y = -0.0207x+1.9969 R^2 = 0.9894	y =14.64x - 6.1725 R^2 = 0.9615	y = -0.0739x+ 2.8002 R^2 = 0.6503

Table 3. Fitting of release data into Zero order, First order and Higuchi equations.

CONCLUSIONS

Ceramic nanoparticles are one of the simplest and novel carriers based on the fundamentals of self assembly. Ceramic nanoparticles for piroxicam were successfully prepared by size enlargement method under reflux conditions. Process and formulation parameters were optimized to get encouraging results, *i.e.* optimum size, shape and percentage of yield. The release of piroxicam was evaluated in 0.1 mol/L hydrochloric acid solution. Plain piroxicam loaded nanoparticles gave enhanced dissolution, *i.e.,* 90% in one hour. Trehalose loaded piroxicam nano particles gave gradual and linear release. Nanosize of the ceramic nanoparticles can be customized further to enhance the delivery of many drugs through oral route.

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