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Voltammetric Behavior of Gemcitabine in Nonaqueous Media and its Analytical Determination in Pharmaceutical Preparation

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ABSTRACT: In this study, simple, fast and reliable linear sweep voltammetry (LSV) method was developed and validated for determination of gemcitabine in pharmaceutical preparation. The proposed method was based on electrochemical oxidation of gemcitabine at platinum electrode in acetonitrile solution containing 0.1 M $LiClO_4$. The well-defined an oxidation peak was observed at 1.27 V. The calibration curve was linear for gemcitabine at the concentration range of 10-60 µg/mL for LSV method. Intra- and inter-day precision values for gemcitabine were less than 2.73, and accuracy (relative error) was better than 3.89%. The mean recovery of gemcitabine was 100.1% for pharmaceutical preparation. No interference was found excipient at the selected assay conditions. The proposed method is highly sensitive, precise and accurate and can be used for the reliable quantitation of gemcitabine in pharmaceutical dosage form.

Keywords: Gemcitabine, Cyclic voltammetry, Linear sweep voltammetry, Validation

INTRODUCTION

Gemcitabine (difluorodeoxycytidine) is a deoxycytidine analogue (Figure 1). It has shown chemotherapeutic activity alone and in combination against a variety of solid tumor types such as ovarian, non-small cell lung, pancreatic, bladder and head/neck squamous cell carcinomas [1-3]. After intravenous administration, gemcitabine is rapidly metabolized in the liver, kidney and other tissues to a noncytotoxic metabolite (2,2´-difluorodeoxyuridine) [4,5].

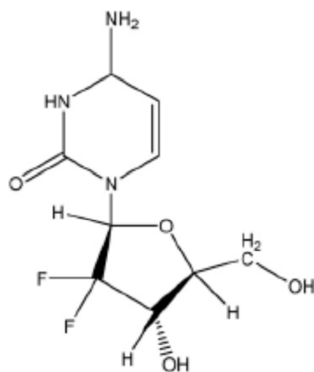

Figure 1: Chemical structure of gemcitabine (difluorodeoxycytidine)

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Several methods have been reported for the determination of gemcitabine including enzyme linked immunosorbance assay (ELISA) [5], ^{19}F-NMR [6], high-performance liquid chromatography tandem-mass spectrometry (HPLC/MS/MS) [7] and derivative-spectrophotometry [8]. Also, HPLC methods have been reported for determining the concentrations of gemcitabine and its metabolite in plasma, cerebrospinal fluid, urine, human carcinoma cells or pharmaceutical preparations [9-14].

The reported methods were influenced by interference of endogenous substances and potential loss of drugs in the re-extraction procedure and involving lengthy, tedious and time-consuming plasma sample preparation and extraction processes and requiring a sophisticated and expensive instrumentation.

The development of a new method capable of determining drug amount in pharmaceutical dosage forms is important. Electroanalytical techniques have been used for the determination of a wide range of drug compounds with the advantages that there are, in most, instances no need for derivatization and that these techniques are less sensitive to matrix effects than other analytical techniques. Additionally, application of electrochemistry includes the determination of

electrode mechanism. Redox properties of drugs can give insights into their metabolic fate or their in vivo redox processes or pharmacological activity [15-18]. Despite the analytical importance of the electrochemical behavior and oxidation mechanism of gemcitabine, no report has been published on the voltammetric study of the electrochemical oxidation of gemcitabine in nonaqueous media. It is well known that the experimental and instrumental parameters directly affect the electrochemical process and voltammetric response of drugs. Consequently, it would be interest to investigate the oxidation process of gemcitabine in aprotic media. Therefore, the goal of this work was the development of new a LSV method for the direct determination of gemcitabine in pharmaceutical preparation without any time-consuming extraction or evaporation steps prior to drug assay. This paper describes fully validated simple, rapid, selective and sensitive procedures for the determination of gemcitabine employing LSV methods the platinum disc electrode. Besides, the method was successfully applied for the quality control of a commercial gemcitabine quantify the drug and to check the formulation content uniformity.

EXPERIMENTAL

Chemical and Reagents

Gemcitabine standard was obtained from Lilly Research Laboratories (Eli Lilly and Company Indianapolis, IN, USA). Acetonitrile (Fluka for HPLC analysis) was purified by drying with calcium hyride, followed by distillation from phosphorus pentoxide. After purification in order to eliminate its water content as much as possible, it was kept over molecular sieves. L**ithium perchlorate** ($LiClO_4$) were purchased from Fluka and used as received without further purification. Pharmaceutical dosage form (Gemzar^R) containing gemcitabine was commercially obtained from pharmacy (Erzurum, Turkey).

Electrochemical Instrumentation

Electrochemical experiments were performed on a Gamry Potentiostat Interface 1000 controlled with software PHE 200 and PV 220. All measurements were carried out in a single-compartment electrochemical cell with a standard three-electrode arrangement. A platinum disk with an area of 0.72 cm^2 and a platinum wire were used as the working and the counter electrodes, respectively. The working electrode was successively polished with 1.0, 0.3 and 0.05 µm alumina slurries (Buehler) on microcloth pads (Buehler). After each polishing, the electrode was washed with water and sonicated for 10 min in acetonitrile. Then, it was immersed into a hot piranha solution (3:1, H_2SO_4, 30% H_2O_2) for 10 min, and rinsed copiously with water. All potentials were reported versus Ag/AgCl/KCl (3.0 M) reference electrode (BAS Model MF-2078) at room temperature. The electrolyte solutions were degassed with purified nitrogen for 10 min before each experiment and bubbled with nitrogen during the experiment.

Preparation of the Standard and Quality Control Solutions

The stock standard solution of gemcitabine was prepared in 0.1 M $LiClO_4$/acetonitrile to a concentration of 100 µg/mL. Working standard solutions were prepared from the stock solution. Standard solutions were prepared as 10-60 µg/mL for LSV. The quality control (QC) solutions were prepared by adding aliquots of standard working solution of gemcitabine to final concentrations of 15, 25 and 55 µg /mL for LSV.

Procedure for Pharmaceutical Preparations

Accurately weighed amount of powder for injection equivalent to 10 mg of gemcitabine was transferred into 100 mL calibrated flask and 50 mL of 0.1 M $LiClO_4$/acetonitrile was added and then the flask was sonicated to 10 min at room tempature. The flask was filled to volume with 0.1 M $LiClO_4$/acetonitrile. The resulting solutions in both the cases were filtered through Whatman filter paper no 42 and suitably diluted to get final concentration within the limits of linearity for the respective proposed methods. The drug content of gemcitabine was calculated from the current potential curve.

RESULTS AND DISCUSSION

Voltammetric Behavior of Gemcitabine

The electrochemical behavior of gemcitabine was investigated at the Pt disc electrode in acetonitrile solution containing 0.1 M $LiClO_4$ as the supporting electrolyte by using cyclic voltammetry (CV). Figure 2 shows a typical cyclic voltammogram of 30 µg/mL gemcitabine recorded under these

conditions for the scan rate of 0.1 V/s. In the anodic sweep, an oxidation peak is seen at about potential of 1.27 V.

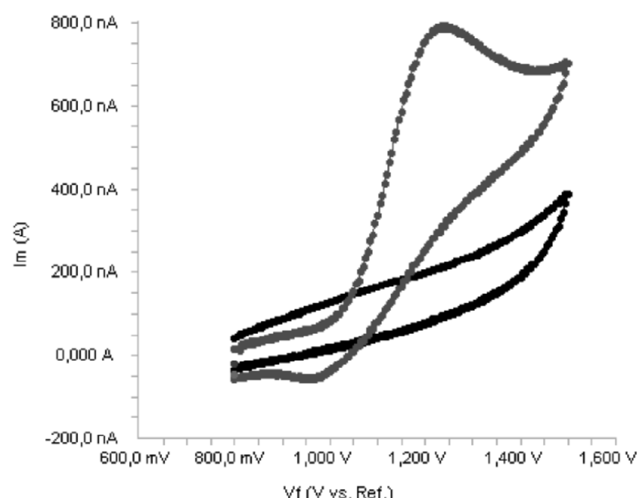


Figure 2: Cyclic voltammogram for the oxidation of 30 μg/mL gemcitabine in acetonitrile containing 0.1 M $LiClO_4$ at Pt disk electrode, scan rate: 0.1 V/s.

In order to gain a deeper insight into the voltammetric waves, the effect of scan rate on the anodic peak currents (I_m) and peak potentials (E_p) was studied in the range of 0.01-1 V/s of the potential scan rates in acetonitrile solution containing 30 μg/mL concentration of gemcitabine (Figure 3).

The representative linear sweep voltammograms obtained at Pt electrode for 30 ìg/mL gemcitabine as a function of the scan rate are presented in Figure 4a,b. However, the plots of logarithm of peak currents versus logarithm of scan rates for 30 μg/mL concentration of gemcitabine display straight lines with 0.46 slope (Figure 4c), which are close to theoretical value of 0.5 expected for an ideal diffusion-controlled electrode process [19]. log I_m-log ν curve is more eligible for this aim, therefore, a diffusional process for peak should be considered. These results suggest that the redox species are diffusing freely from solution and not precipitating onto the electrode surface. The reason for this behavior may be due to the solubility of the intermediate species in acetonitrile or poor adherence of products on the electrode surface.

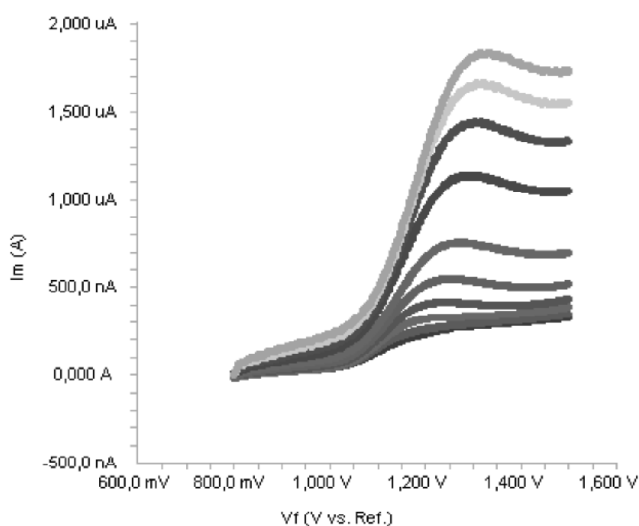


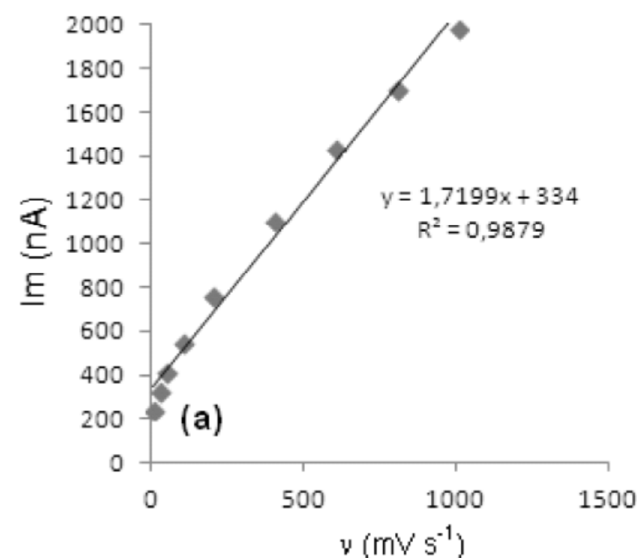
Figure 3: Linear sweep voltammograms for the oxidation of 30 μg/mL gemcitabine in acetonitrile containing 0.1 M $LiClO_4$ as a function of scan rate.

As shown in Figure 3, the oxidation peak potential (E_{pa}) for peaks shift toward more positive values with increasing scan rate. The relationship between the peak potential and scan rate is described by the following equation [20],

$$E_{pa} = E^{0'} + RT/[(1-\alpha)n_aF][0.78 + \ln(D^{1/2}k_s^{-1}) - 0.5\ln RT/[(1-\alpha)n_aF]] + RT/[(1-\alpha)n_aF]/2\ln \nu$$

and from the variation of peak potential with scan rate an_a can be determined, where a is the transfer coefficient and n_a is the number of electrons transferred in the rate determining step. According to this equation, the plots of the peak potentials versus ln n for oxidation peak show linear relationship (Figure 5).

The slope indicate the value of αn_a is 1.0 for peak. Also, this value obtained indicate the total irreversibility of the electron transfer processes. This result show that the chemical step is a fast following reaction coupled to a charge transfer.



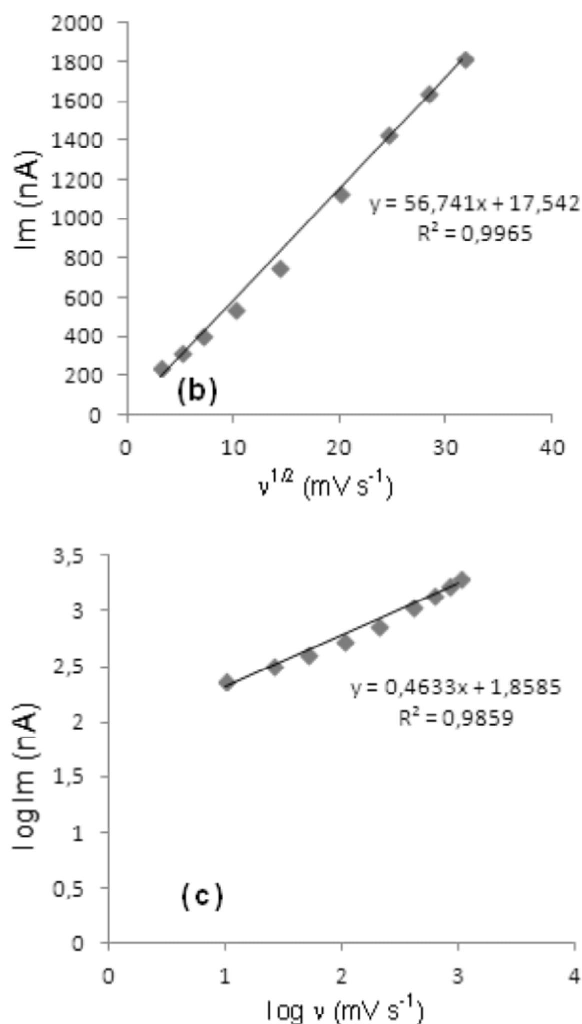


Figure 4(a-c): Dependence of peak current on the scan rate (30 µg/mL).

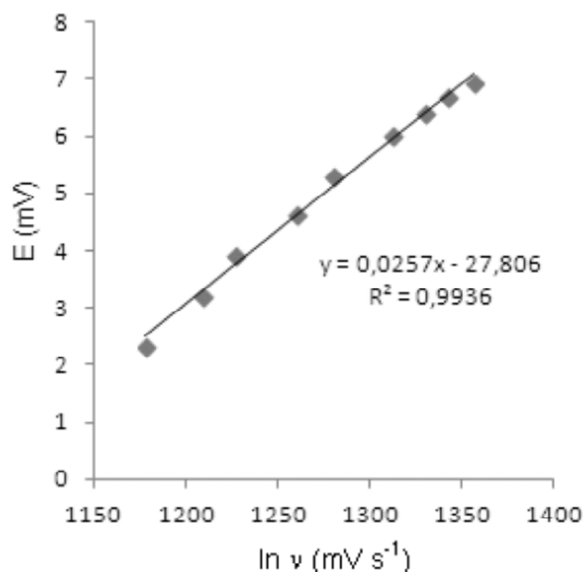


Figure 5: Dependence of anodic peak potentials of voltammetric peak for the oxidation of 30 µg/mL gemcitabine on the scan rate.

Validation of the Method

The validation was carried out by establishing specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), stability, recovery according to ICH Q2B recommendations [21, 22].

Specificity

The effects of common excipients and additives were tested for their possible interferences in the assay of gemcitabine. The simulated and placebo samples were prepared and analyzed. It has not been determined any interference of these substances at the levels found in dosage forms. Excipient that was used in this preparation was the most commonly used by the pharmaceutical industry. The specificity of the method was investigated by observing any interference encountered from the common tablet excepients such as titanium dioxide, **sodium chloride,** talc, lactose, starch, and magnesium stearate. These excepients did not interfere with the proposed method.

Linearity

Standard solutions were prepared as 10-60 µg/mL (10, 20, 30, 40, 50 and 60) for LSV, (Figures 6).

Calibration curve was constructed for gemcitabine standard by plotting the concentration of compound versus peak current responses. The calibration curves were evaluated

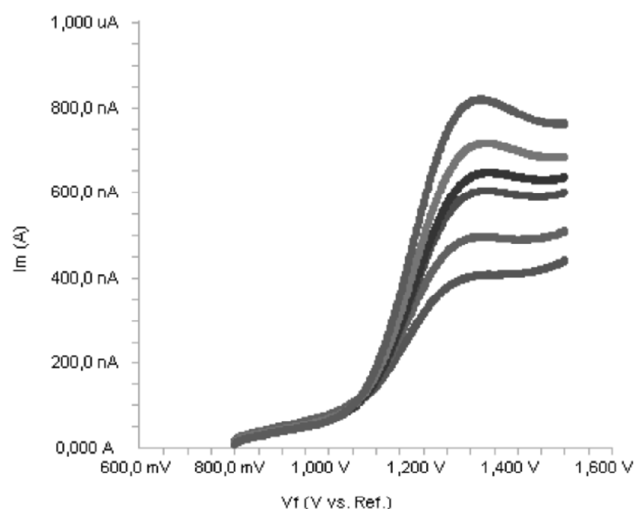


Figure 6: Linear sweep voltammograms for different concentrations of gemcitabine in acetonitrile solution containing 0.1 M $LiClO_4$ (10, 20, 30, 40, 50 and 60 mg/mL).

by its correlation coefficients. The correlation coefficients *(r)* of all the calibration curves were consistently greater than 0.99. The linear regression equations were calculated by the least squares method using Microsoft Excel® program and summarized in Table 1.

Table 1
Linearity of Gemcitabine

Method	*Range (µg/mL)*	*LR*[a]	S_a	S_b	R^2	*LOD*	*LOQ*
LSV	10-60	y=8.1571x+323.33	7.410	0.024	0.9921	3.00	9.00

[a] Based on three calibration curves, LR: Linear regression, Sa: Standard deviation of intercept of regression line, Sb: Standard deviation of slope of regression line, R^2: Determination of correlation, y: Peak current, x: Gemcitabine concentration (mg/mL), LOD: Limit of detection, LOQ: Limit of quantification

Accuracy and Precision

Accuracy of the assay methods was determined for both intra-day and inter-day variations using the quality control (QC) samples. Precision of the assay was determined by repeatability (intra-day) and intermediate precision (interday). Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time that was evaluated by assaying the QC samples during the same day. Intermediate precision was assessed by comparing the assays on different days (2 days). The intra-day accuracy ranged from 1.68% to 2.45% and precision from 2.04% to 2.73% (Table 2). The results obtained from intermediate precision (inter-day) also indicated a good method precision. All the values were within the acceptance criteria of 2.73%.

Table 2
Precision and Accuracy of Gemcitabine

Method	*Added (µg/mL)*	*Found±SD*[a] *(µg/mL)*	*Intra-day Accuracy*	*Precision RSD%*[b]	*Found±SD (mg/mL)*	*Inter-day Accuracy*[c]	*Precision RSD%*[b]
LSV	15	15.20±0.372	1.33	2.45	15.33±0.419	2.20	2.73
	25	24.89±0.418	-0.44	1.68	25.16±0.597	0.64	2.37
	55	52.86±0.972	-3.89	1.84	53.07±1.082	-3.51	2.04

[a] SD: Standard deviation of six replicate determinations, [b] RSD: Relative standard deviation,
[c] Accuracy: (%relative error) (found-added)/addedx100

Limits of Detection (LOD) and Quantification (LOQ)

The LOD and LOQ of gemcitabine by the proposed method was determined using calibration standards. LOD and LOQ values were calculated as 3.3 σ/S and 10 σ/S, respectively, where S is the slope of the calibration curve and $\acute{o}$ is the standard deviation of y-intercept of regression equation (n=6) [22]. The LOD and LOQ values of the methods were summarized in Table 1.

Stability

To evaluate the stability of gemcitabine, standard solutions were prepared separately at concentrations covering the low, medium and higher ranges of calibration curve for different temperature and times. These solutions were stored at room temperature, refrigeratory (4 °C) and frozen (-20 °C) temperature for 24 h and 72h. Stability measurements were carried out with LSV method. The results were evaluated comparing these measurements with those of standards and expressed as percentage deviation and insulin was found as stable at room temperature, 4 and -20 °C for at least 72h (Table 3).

Recovery

To determine the accuracy of the LSV method and to study the interference of formulation additives, the recovery was checked as three different concentration levels. Analytical recovery experiments were performed by adding known amount of pure drugs to pre-analyzed samples of

Table 3
Stability of Gemcitabine in Solution

Stability (%)		Room temperature stability, (Recovery % ± RSD)		Refrigeratory stability, +4°C (Recovery % ± RSD)		Frozen stability, - 20°C (Recovery % ± RSD)	
Method	*Added(mg/mL)*	*24 h*	*72 h*	*24 h*	*72 h*	*24 h*	*72 h*
LSV	15	96.7±2.16	98.4±2.46	98.2±0.74	97.4±2.32	98.3±2.28	102.1±1.86
	25	98.8±1.92	99.1±2.41	101.2±3.09	99.8±1.06	96.4±2.21	99.4±2.92
	50	100.8±3.64	98.6±2.65	99.3±2.18	98.6±1.52	97.7±3.16	99.7±1.68

SD: Standard deviation of six replicate determinations

commercial drug form. The recovery values were calculated by comparing concentration obtained from the spiked samples with actual added concentrations. These values are also listed in Table 4.

Table 4
Recovery Values of Gemcitabine in Pharmaceutical Preparation

Method	*Gemzar*	*Added (mg/mL)*	*Found±SD (mg/mL)*	*Recovery (%)*	*RSD [a] (%)*
LSV	(200 mg)	5	4.97±0.201	99.4	4.04
		15	15.10±0.517	100.7	3.42
		35	33.85±0.941	96.7	2.78

SD: Standard deviation of six replicate determinations, RSD: Relative standard deviation,

[a]Average of six replicate determinations

The official methods for determination of gemcitabine in pharmaceutical dosage forms are prescribed in the United States Pharmacopoeia (USP). USP 27 prescribes HPLC method for determination of gemcitabine in powder form, using a reversed phase column with particle size 5 ìm, length 4.2 mm x 25 cm, sodium phosphate and phosphoric acid as mobile phase, UV detection at 275 nm and flow rate 1.2 mL/min.

Different liquid chromatographic methods have been reported for determination of gemcitabine especially in human plasma, serum in literature. But, no method has been developed on the validated voltammetric method for estimation of gemcitabine in lyophilized powder dosage form. Typical bio analytical sample preparation techniques such as extraction into an organic solvent one not effective because of the extremely hydrophilic nature of the compound.

Also, the proposed method was compared with HPLC method in literature [23]. According to the statistical comparison (Student t-test) of the results there is no significant difference between proposed and reference methods (Table 5).

Table 5
Comparison of the Proposed and Reported HPLC Method [23] for Determination of Gemcitabine

Method	*Commercial Preparation*	*n*	*Found ± SD (mg)*	*Recovery (%)*	*RSD[a] (%)*	*t-test*
LSV	Gemzar (200 mg)	6	200.1 ± 1.76	100.05	0.88	t_t=1.78
HPLC		6	198.54± 1.86	100.18	0.94	t_c=1.24

n: Number of determination, SD: Standard deviation of six replicate determinations,

RSD[a]: Relative standard deviation, t_c: calculated *t*-value, t_t: tabulated *t*-value,

Ho hypothesis: no statistically significant difference exists between two methods,

$t_t > t_c$: Ho hypothesis is accepted (P > 0.05)

In conclusion, the electrochemical behavior of gemcitabine has been studied in nonaqueous media by CV and LSV voltammetry methods. Besides, in the present report, a simple, rapid, sensitive, reliable, specific, accurate and precise LSV method for the determination of gemcitabine in pharmaceutical preparation was developed and validated. The method described has been effectively and efficiently used to analyze gemcitabine pharmaceutical preparations without any interference from the pharmaceutical excipients. The voltammetric run time of 1 min allows the analysis of a large number of samples in a short period of time. Therefore, the method can be used effectively without separation for routine analysis of gemcitabine in pure form and its formulations.

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