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# IMPROVED AQUEOUS DISSOLUTION OF CLOFAZIMINE FROM COEVAPORATES USING POLYVINYLMETHYL ETHER / MALEIC ANHYDRIDE COPOLYMER

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## ABSTRACT

The water-insoluble antileprotic drug clofazimine, was solubilized by solid dispersion in polyvinylmethyl ether/maleic anhydride copolymer. Dissolution tests in acidic, neutral and alkaline media showed that the dispersion (coevaporate) exhibited superior dissolution characteristics, compared to the pure compound or the physical admixtures of the drug and the polymer. Dissolution tests also revealed that the release of clofazimine from the coevaporate in the test solutions, was of zero-order and that rate and extent of release increased with polymer concentration in the coevaporate. However, the lag-time prior to drug release also increased with polymer concentration. The rate of release was enhanced in the alkaline medium.

UV-visible and infra red spectra and also thin layer and high performance liquid chromatograms taken after the coevaporate preparation and several times during storage, indicated no apparent physicochemical change in clofazimine in the coevaporate. Accelerated stability studies of the coevaporate during a 12-week period at $25^0$, $37^0$, and $50^0$C indicated that negligible amount of clofazimine had degraded during the storage period. Kinetically the apparent loss of clofazimine in the coevaporate is indistinguishable between zero-order and first-order.

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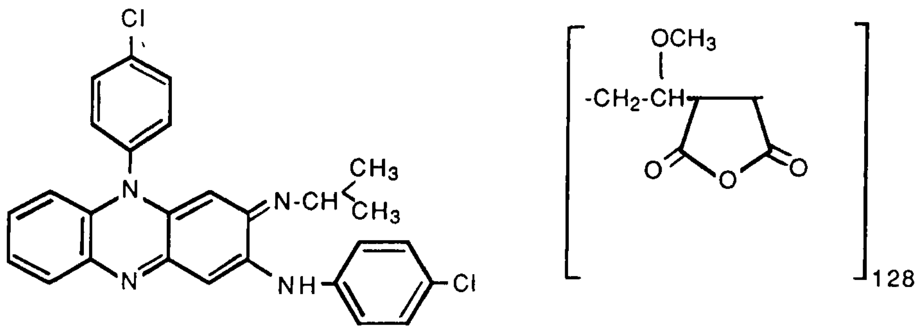
## INTRODUCTION

Clofazimine, [3-(p-chloroanilino)-10-(p-chlorophenyl)-2,10-dihydro-2-(isopropylimino) phenazine] (CLF, FIG.I a) is one of the three principal drugs recommended by the World Health Organization (WHO) in a combination therapy for the treatment of leprosy[1]. Unlike other classical antileprotic drugs, so far the reports of *Mycobacterium leprae* resistance to CLF are rare[2]. Besides its antileprotic activity, it has also been reported to be effective in Buruli skin ulcers caused by *Mycobacterium ulcerans*[3], pyoderma gangrenosum[4,5] and generalised granuloma annulare[6]. Currently, the drug is being tried in patients with acquired immune deficiency syndrome (AIDS) complicated by *Mycobacterium avium* infection[7].

CLF is lipophylic and only sparingly soluble in water. Currently, it is marketed as a soft gelatin capsule, containing micronised drug suspended in an oil-wax base (Lamprene®,Ciba Giegy). Although the extent of absorption of CLF from Lamprene® is reported to be as high as 70% of the administered dose[8], it is generally recognized that the rate and extent of absorption are low and erratic and differ from patient to patient [9]. It seems that in patients receiving 100 - 200 mg of clofazimine daily, it takes 50 days to reach bactericidal action against *Mycobacterium leprae*[10]. It is also reported that upto 50% of the administered dose of CLF is recovered unchanged from faeces[11]. It is unclear what portion of this represents unabsorbed drug or unchanged drug that has been excreted by the enterohepatic route. It is likely that the low systemic availability and erratic absorption behaviour of the drug, may be due to its poor aqueous solubility and therefore, dissolution may be the rate-determining step in absorption.

Soft gelatin capsule dosage form *per se* is fraught with many production and storage problems. Aside from the inherent cumbersome and costly production methods requiring stringent quality control procedures, there is always the problem of prolonged disintegration time with oil-filled soft gelatin capsules and the potential for leakage of contents from the capsule seam. In case of CLF in an oily base, such leakage invariably results in aesthetically unacceptable product.

The purpose of this study is to impove the aqueous dissolution of CLF and hence, its systemic availability, through solid dispersion. It is anticipated that the study would lead to the formulation of an alternative solid dosage form.



(a) Clofazimine

(b) GAN-119

FIGURE I

Solid dispersions of poorly soluble drugs have been reported to result in improved dissolution and bioavailability[12-16]. Polyvinylalkyl ether/maleic anhydride copolymers and their esters (Gantrez® polymers) have been used in pharmaceutical formulation as an adjuvent in tablet and microcapsule coating and as tablet matrix material[17-20]. The clinical effictiveness of 2%Gantrez in combination with 0.2-0.5%of the antibacterial agent triclosan, in dentifrices, in reducing salivary bacterial count, dental plaque and gingivitis has been demonstrated in a number of studies[21-27].The use of Gantrez polymers in solid dispersion with griseofulvin has also been reported[28].

In this paper, we report the use of polyvinylmethyl ether/maleic anhydride copolymer (GAN-119) in the preparation of CLF dispersion and the improved dissolution characteristics of the dispersion.

## MATERIALS

GAN-119, (FIG. I b, GAF Corporation, New Jersey, U.S.A.) and CLF (Astra-IDL Limited, Bangalore, India) were used as received.

Solvents and reagents used in the project were of analytical grade.

## METHODS

### Preparation Of CLF–GAN-119 Coevaporate

Generally solid dispersions are accomplished by 'melt method', in which an intimate mixture of an aqueous-soluble carrier and insoluble drug is melted and rapidly cooled to solidify. In the present project, because of the thermal decomposition of GAN-119 at the apparent melting point (210 $^0$C), the melt method was inapplicable. Therefore the dispersion of CLF in GAN-119 was achieved by the 'solvent method'.

CLF and GAN-119 were separately weighed and individually dissolved in acetone. The acetone solutions were mixed and allowed to stand for five minutes. The solvent was evaporated under reduced pressure with the aid of flash evaporator (Brinkmann Rotavapor-R, Switzerland). The flaky coevaporate was powdered by trituration and dried in a desiccator under vacuum, over silica gel. Finally, they were sifted through 85 mesh sieve (Endecotts Filters limited, England) and stored in tightly closed amber colored bottles, in a desiccator. Coevaporates containing different ratios of CLF and GAN-119 were prepared and the amount of drug in each was ascertained by assay.

### Preparation Of Physical Admixture Of CLF And GAN-119

Similar compositions of CLF and GAN-119 as used in the preparation of coevaporates were taken in a glass beaker and intimately mixed using a stainless-steel spatula. The homogenous mixture was similarly passed through the 85-mesh sieve and stored as prescribed for the coevaporate.

### Determination Of CLF In *In Vitro* Aqueous Sample

A simple nonextractive method was developed for routine determination of CLF concentration in aqueous samples from *in vitro* systems. From a stock solution of CLF in 6N hydrochloric acid (HCl), various dilutions of known CLF concentrations were prepared using 4N HCl. The absorbances of the diluted CLF solutions were measured in a spectrophotometer (Pye-Unicam SP 8-100, England) at 535nm using 4N HCl as blank. A calibration curve was prepared, relating absorbance (ordinates) with known concentrations of CLF (abscissae). Equation of best fit was also derived.

In the determination of an unknown CLF concentration in an *in vitro* sample, the filtered aliquot was suitably acidified using HCl, so as to maintain the final acid strength between 3--6 N and the absorbance was determined. The CLF concentration was then calculated using the calibration curve.

### Dissolution Of CLF From Coevaporate And Physical Admixture

Dissolution tests were performed using a U.S.P. Type II apparatus (Vanderkamp 60, U.S.A.), using three solvent systems of different pH. The acidic medium (dilute HCl, N/10, pH 1.2), was prepared by diluting 8.7 ml of concentrated HCl to 1000 ml, with distilled water. The pH was checked and if necessary was adjusted to 1.2 using 1N HCl or water. The alkaline medium (dilute sodium hydroxide (NaOH), N/1500, pH 10.0), was prepared by suitably diluting N/10 NaOH with water. Final pH was checked and if necessary was adjusted to 10.0 using N/100 NaOH or distilled water. The buffer medium (0.5 mM, pH 7.5) was freshly prepared each time by suitably diluting 0.05 M potassium phosphate monobasic solution, with distilled water. The final pH was checked.

Coevaporate or physical admixture sample containing about 2.5 mg of CLF was exactly weighed and sprinkled on 1000 ml of solvent surface, which was stirred at 50 rpm at 37±0.5$^0$C. At suitable time points over a period of 120 minutes, aliquots of the dissolution medium were withdrawn using a disposable plastic syringe (Becton-Dickinson and company, U.S.A.), fitted with a filtration assembly, to prevent undissolved CLF from entering the sample. Fresh medium equilibriated to 37$^0$C, was added to replenish the sampled volume. The filtrate was further passed through 4.5-5 ASTM sintered glass filter, fitted with 0.45μ membrane filter (Millipore Corporation, U.S.A.). After two-fold dilution with 6N HCl, absorbance of the sample was measured at 535 nm. The concentration of dissolved CLF in the dissolution medium was calculated with the aid of the calibration curve. Reported values are the mean of at least, two determinations.

### Dissolution Of CLF From Fixed Surface Area Of Compressed Coevaporate

To determine the comparative dissolution rate of CLF from coevaporates, a fixed surface area (0.985 cm$^2$) of compressed coevaporate was exposed to one litre of acidic dissolution medium under same experimental conditions as for the powder dissolution. The compressed sample was made by subjecting approximately 150 mg of the coevaporate of known composition, contained in a 11.2 mm diameter steel die and punch set, to a 16000-lbs. force for 3 minutes, with the aid of a press (Carver Laboratory Press, U.S.A.). The compressed sample was then partially embedded in molten wax (about 45$^0$C), such that only one full lateral surface free of wax, was exposed to the dissolution medium.

At specified time points, 5 ml aliquots of bulk solution were withdrawn, filtered, diluted with 6N HCl and assayed spectrophotometrically for CLF content. The dissolution medium volume was maintained constant by replenishing the sample volume by fresh medium, equilibriated to 37$^o$C.

The cumulative amount of CLF dissolved at each time point was calculated and corrected for the amount of dissolved CLF in the sample removed according to the equation:

$$Q_t = [ Q_i + \{ V_s . Q_{(t-1)} \} / V_b ]. \qquad (1)$$

In equation (1), $Q_t$ is the cumulative amount of CLF dissolved up to time, t; $Q_i$ is the amount of CLF in the dissolution medium determined at time t; $Q_{(t-1)}$ is the amount of CLF dissolved at the previous sampling time, (t-1). $V_s$ and $V_b$ are respectively the volume of sample and dissolution fluid.

The percentage of total CLF in the compressed sample dissolved per unit area was calculated at each time point and fitted to the following rearranged form of the integrated Noyes-Whitney equation, assuming sink conditions:

$$100. [ (Q_t / Q_0) /S] = (D . Cs / h). t \qquad (2)$$

In equation (2), $Q_0$ is the total (original) amount of CLF in each compressed sample. D is the diffusion coefficient, Cs is the intrinsic solubility of CLF in medium under experimental conditions and h is the thickness of the stagnant (diffusion) layer.

Equation (2) may be simplified to

$$100. [ (Q_t / Q_0) / S ] = K_d . t \qquad (3)$$

where $K_d$ = ( D.Cs / h ), is a constant under a given experimental condition and represents the apparent dissolution rate constant. $K_d$ was calculated as the slope of the linear plot of $[\{Q_t/Q_0\}/S]100$ (ordinates) *versus* time,t.

## Stability Testing Of CLF in Coevaporates

The stability testing of CLF in coevaporate was performed with two objectives. Firstly, it was to ascertain if CLF remained chemically intact following treatment during preparation of the coevaporate. Secondly, it was necessary to determine whether or not CLF in coevaporate maintained essentially the same physicochemical characteristics during prolonged storage period under different temperature conditions. In pursuance of these objectives, certain physico-chemical-property indicators were monitored over a 12-week period, on a 1:10 (CLF:GAN-119) coevaporate. These included thin layer chromatography (TLC), high performance liquid chromatography (HPLC), ultra violet and infra red spectroscopy . Additionally, the amount of CLF remaining in 1:10 coevaporate during storage at $25^0$ (room temperature), $37^0$ and $50^0$C was monitored periodically during the period.

## Thin Layer Chromatography (TLC)

Samples of pure, untreated CLF and coevaporate were seperately dissolved in acetone. Precoated silicagel plates ( F254, E.Merck, U.S.A.) were conditioned by pre-running in chloroform-methanol (1:1 by volume) and dried. The plates were spotted with the test solutions then developed in toluene-acetic acid-1-propanol (58:39:3 by volume). The spots were then visualized by spraying the plates with 50 % V/V sulphuric acid.

## High Performance Liquid Chromatography (HPLC)

A reported HPLC method [29], was adapted to detect CLF in *in vitro* aqueous media. The liquid chromatograph system comprised a Waters ® HPLC (model 440) equipped with 6000A pump and U6K universal injector. A fixed wave length (280 nm) UV detector with a chart recorder (Omni Scribe,Houston Instruments, U.S.A.) was used. The analytical column used was μ Bondapack$C_{18}$ (300 mm x 3.9 mm I.D., Waters Assoc., U.S.A.).

In the modified method the mobile phase consisted of 50% tetrahydrofuran (THF) in 0.5% acetic acid containing 2.5 mM hexane sulfonic acid and was delivered isocratically at 1.5 ml/min. Samples of pure, untreated CLF and coevaporate were dissolved seperately in mobile phase, and 25 µl were injected. The chromatograms were obtained from time to time during the 12-week period and were compared.

### Ultra Violet-Visible Spectroscopy

A 3–5 µg/ml solution of pure untreated CLF in 4N HCl was scanned in the spectrophotometer between 600 and 250 nm. A solution of the freshly prepared coevaporate containing the same concentration of CLF in 4N HCl was scanned similarly, using as blank, a solution of GAN-119 in 4N HCl, containing an equivalent concentration of the polymer, as in the coevaporate solution.

Thereafter, the procedure was repeated with the incubated samples periodically in the twelve week period during which the stability was monitored.

### Infra Red (I.R.) Spectroscopy

Infra red spectrum of pure CLF and that extracted from the coevaporate were obtained using Perkin Elmer-197 (England) instrument, set at 8 minutes scan time and slit 2. Potassium Bromide (KBr, I.R. grade) pellets were made, having 0.5% w/w of test sample.

CLF was extracted with chloroform from aqueous solution of coevaporate. The chloroform extract was washed with water, dried over anhydrous sodium sulfate and evaporated using flash evaporator at $30^0$C. The residue was dried in vacuum desiccator. The I.R. spectrum of the residue was compared with that of pure CLF.

### Determination Of Intact CLF In Coevaporate During Accelerated Stability Study

1:10 coevaporate samples were incubated at $25^0$(room temperature), $37^0$ and $50^0$ ($\pm 0.5^0$C). At specified time points aliquots of each sample were removed and equilibrated to room temperature. Weighed amounts of aliquots were dissolved in 4N HCl and respective absorbances were measured at 535 nm, as described earlier. The concentration of CLF in each solution and hence, the amount of unchanged CLF remaining in coevaporate were determined with the aid of calibration curve, prepared using standard solutions of pure CLF. The samples were also subjected to TLC, HPLC, UV-visual scan tests and dissolution in acidic medium.

The kinetics of decomposition were determined by plotting the percentage of CLF remaining in the coevaporate *versus* time, according to the following equations assuming zero-order and first-order decomposition process respectively

$$C_t = C_0 - K_0.t \qquad (4)$$

$$\mathrm{Log}\ C_t = \mathrm{Log}\ C_0 - K_1.t / 2.303 \qquad (5)$$

In equations (4) and (5) $C_t$ is the percentage of CLF in the coevaporate at any time t after preparation; $C_0$ is the initial percentage of CLF in coevaporate on the day of preparation; $K_0$ and $K_1$ are respectively, the zero-order and the first-order rate constants associated with the decomposition process. The rate constants were calculated from the slopes of the percentage remaining--time plots.

Shelf lives ($t_{95\%}$), representing the time required for 5% decomposition of CLF in the coevaporate were calculated according to the following equations, assuming zero-order decomposition (Eq.5) or first order decomposition (Eq.7) :

$$t_{95\%} = 0.05\ C_0 / K_0 \qquad (6)$$

$$t_{95\%} = 0.05 / K_1 \qquad (7)$$

## RESULTS

### Physical Characteristics Of Coevaporates

The pulverized and dried coevaporate was dark red in color, non-sticky, free-flowing and showed no tendency of hygroscopy or clumping under the storage conditions. There was no apparent change in these physical characteristics during the 12 week storage period. The yield was more than 95 % w/w.

### Ultra Violet-Visible Spectrophotometric Determination

Protonation of CLF is pH dependent[30]. Solutions of CLF in dilute HCl with normality between 3--6 were found to have absorbance maxima at 535 nm. Over the concentration range of 0.02 and 6 µg/ml in 3--6 N HCl, solutions of CLF exhibited linear absorbance with concentration as demanded by Beer-Lambert's law (FIG.II). By linear regression analysis of the mean absorbance - concentration data, the linear equation of best fit determined was, y =0.09 x - 0.002, in acidic medium (3–6 N HCl).

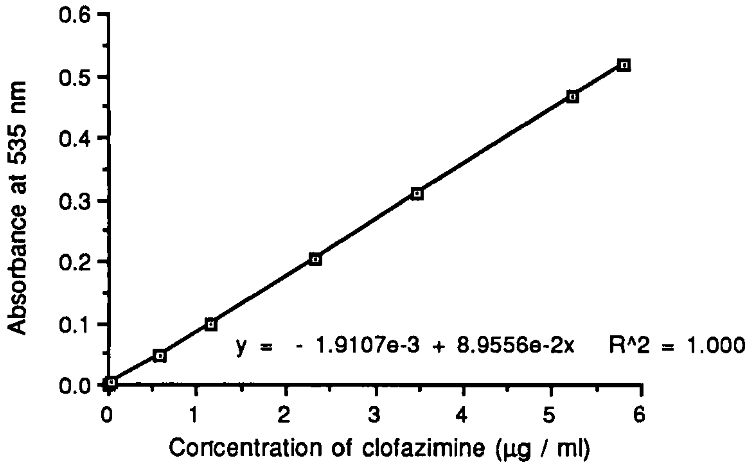


FIGURE II

Calibration curve of clofazimine

Replicate determination (n=5) on different occasion showed high reproducibility (coefficient of variation = 0.5%).

Each time *in vitro* CLF samples were to be analysed, the equation of best fit to the calibration curve was determined and was used to estimate unknown CLF concentrations in the samples.

## Dissolution Of CLF In Coevaporates

Dissolution profiles of CLF in the powdered coevaporates of different composition in acidic medium (pH1.2), buffer medium (pH7.5) and alkaline medium (pH 10.0) are shown in Figure III, a, b and c. It is apparent that increasing GAN-119 concentration up to 95%, in the coevaporate resulted in an increased solubility of CLF. Further increase in GAN-119 concentration, however, did not cause any precipitation.

In the acidic medium, 20 and 84% respectively, of total CLF were released from 1:1 (50% CLF) and 1:5 (17%CLF) coevaporates in two hours. In the same medium, the maximum amount of CLF released from 1:10 (9%CLF), 1:15 (6%CLF) and 1:20

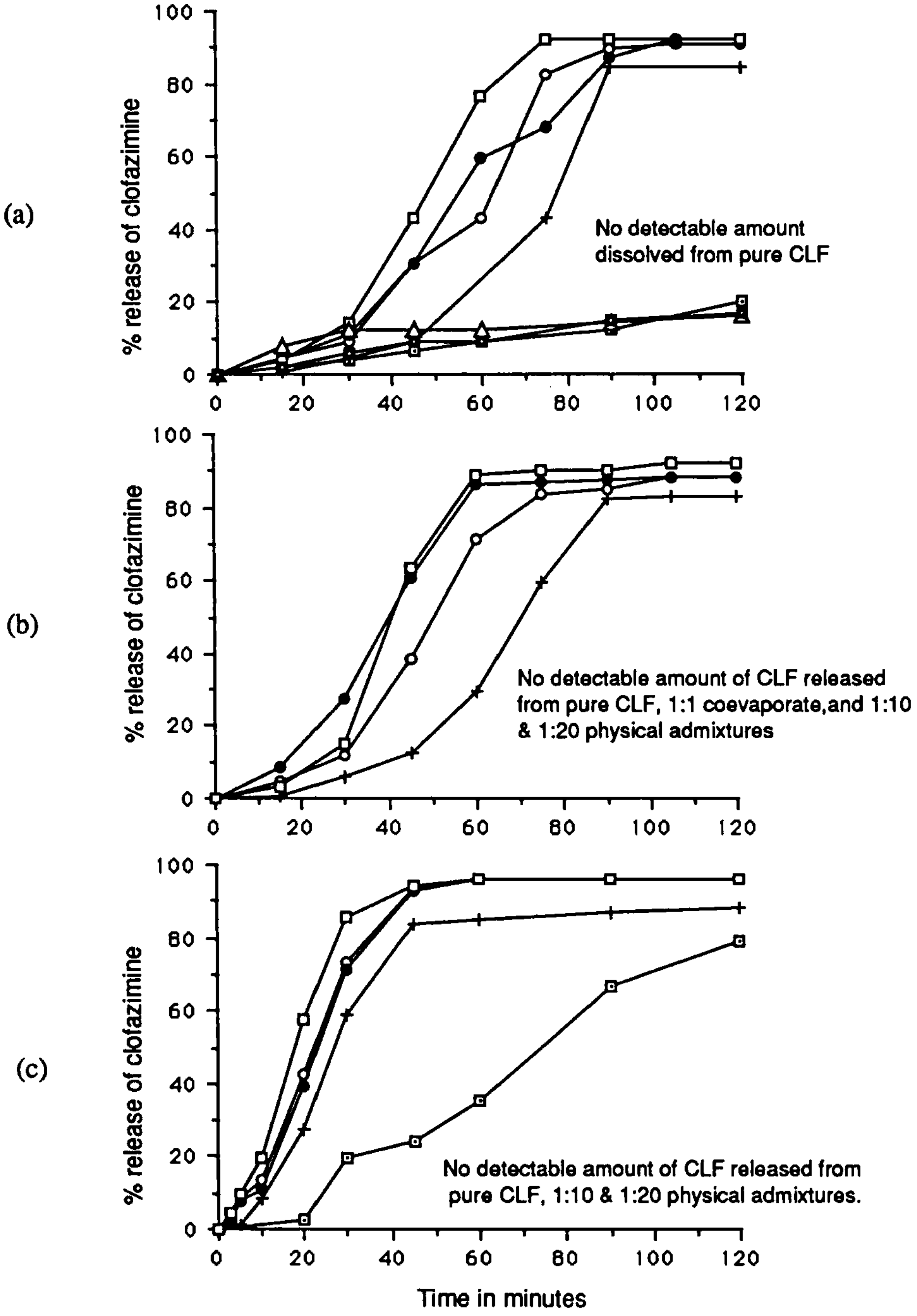


FIGURE III
Dissolution profiles of powder samples of coevaporates and physical admixtures,
(a) in acid medium (pH1.2), (b) in buffer medium (pH7.5), (c) in alkaline medium (pH10.0)
Legends: Coevaporates: ▣ 1:1, + 1:5, o 1:10, ● 1:15, □ 1:20; Phy.ad.: Δ 1:10, ■ 1:20

(5%CLF) coevaporates were similar (90-93%), which occured in about 90 minutes. The different peak concentration times indicate differences in release rates of CLF from coevaporates with different composition. In acidic pH, negligible amount (below the analytical sensitivity) of CLF was detected from pure, untreated CLF. However, about 16--17% was released from 1:10 and 1:20 physical admixtures.

In buffer medium, 1:5 and 1:20 coevaporates respectively, released 83 and 93% of total CLF present. The amount released from 1:10 and 1:15 coevaporates were similar (89%). These peak concentrations were recorded in 105 minutes. In this medium also, it is apparent that release rate of CLF was different with each composition (FIG. III, b). There was no detectable concentration of CLF released from 1:1 coevaporate.

In the alkaline medium, 1:1 and 1:5 coevaporates released 80 and 88% of total CLF respectively, in 120 minutes (FIG. III, c). On the other hand, 96% of CLF was released from all the other three, 1:10, 1:15 and 1:20 coevaporates, in 60 minutes. Here again, it is evident that release rates were different for different coevaporate compositions.

There was no detectable concentration of CLF dissolved from pure CLF, and any of the physical admixtures, in both buffer and alkaline media.

To elucidate the kinetics of drug release from these coevaporates, the percentage of CLF, remaining to be released from coevaporates, as a function of time was examined. The profiles suggest zero-order release of CLF. However, at lower polymer concentration (≤50%) the release is apparently first-order in acidic medium. To confirm these observations, the percentage remaining to be released at anytime t, was fitted separately to the following equations

$$[X_\infty - X_t] = X_\infty - K_0 . t \qquad (8)$$

$$\ln [X_\infty - X_t] = \ln X_\infty - K_1 . t \qquad (9)$$

indicating zero-order (equation 8) or first-order (equation 9) release. In the above equations $X_t$ and $X_\infty$ represent respectively, the percentage of CLF released at any time t and at infinite time, under experimental conditions. $K_0$ and $K_1$ are the release rate constants. In both linear equations the ordinate intercept represents $X_\infty$. The calculated (predicted) values and the experimental values are summarised in Table I.

TABLE I

Release Of Clofazimine From Coevaporate Powder Samples

| Parameter | | Coevaporate Composition | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1:1 | | 1:5 | | | 1:10 | | | 1:15 | | | 1:20 | |
| | | Acidic | Alkaline | Acidic | Buffer | Alkaline | Acidic | Buffer | Alkaline | Acidic | Buffer | Alkaline | Acidic | Buffer | Alkaline |
| ZERO-ORDER | Ko (%/min) | 0.154 | 0.618 | 0.211 | 0.473 | 1.662 | 0.750 | 1.176 | 2.237 | 0.963 | 1.505 | 2.208 | 1.280 | 1.588 | 2.341 |
| | Intercept* | 20.42 | 81.90 | 85.34 | 87.58 | 90.98 | 95.75 | 98.42 | 98.54 | 100.18 | 96.91 | 99.31 | 103.31 | 105.98 | 94.82 |
| | Corr.Coeff. | 0.99 | 0.92 | 0.93 | 0.84 | 0.95 | 0.92 | 0.88 | 0.98 | 0.88 | 0.96 | 0.98 | 0.90 | 0.89 | 0.95 |
| FIRST-ORDER | K i (1/min) | 0.010 | 0.009 | 0.003 | 0.007 | 0.064 | 0.011 | 0.025 | 0.077 | 0.016 | 0.058 | 0.071 | 0.027 | 0.052 | 0.091 |
| | Intercept* | 20.95 | 82.02 | 85.47 | 89.08 | 133.59 | 99.04 | 116.87 | 140.18 | 108.32 | 169.79 | 136.58 | 124.91 | 170.73 | 139.67 |
| | Corr.Coeff. | 0.97 | 0.84 | 0.93 | 0.80 | 0.87 | 0.89 | 0.77 | 0.89 | 0.81 | 0.75 | 0.89 | 0.78 | 0.75 | 0.95 |
| Total release (experimental) | | 20.08 | 79.12 | 84.30 | 83.08 | 88.44 | 90.84 | 88.44 | 96.00 | 92.48 | 88.44 | 96.00 | 92.48 | 92.48 | 96.00 |

* Represents % eventually released

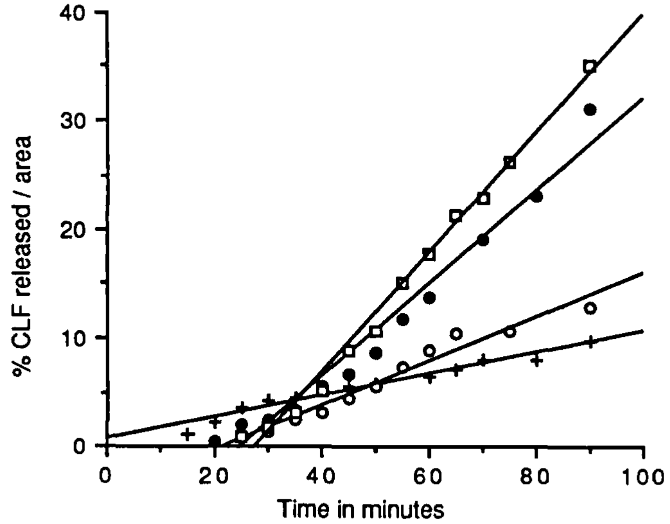


FIGURE IV

Dissolution profiles of clofazimine from fixed surface of compressed coevporates in acidic medium (pH 1.2).Legends:Coevaporates + 1:5, o 1:10, ● 1:15, □ 1:20

Estimates of $X_\infty$ obtained with equation (8) were comparable to experimental values and suggest zero order release. The analysis also indicated increasing release rate constant, with increasing polymer concentration.

To further investigate the dissolution characteristics of coevaporates, release of CLF from a fixed surface area was studied in the acidic medium. Figure (IV) represents composite plots of the percentage of total CLF released per unit surface area versus time, according to equation (3). These plots suggest zero-order release of CLF from the compressed surface. They also show increasing lag time prior to drug release, with increasing polymer composition. This observation was confirmed by regression analysis data (Table II). The analysis also indicates that, as with powder coevaporate, the apparent dissolution rate constant ($K_d$) also increases with increasing polymer content in the formulation.

TABLE II

Dissolution Of Clofazimine From Fixed Surface Area, In Acidic Medium (pH 1.2)

| Parameters | Coevaporate composition | | | |
|---|---|---|---|---|
| | 1:5 | 1:10 | 1:15 | 1:20 |
| Apparent dissolution rate constant $K_d$ (%cm$^{-2}$ min$^{-1}$) | 0.102 | 0.206 | 0.426 | 0.551 |
| Lag time (minutes) | 6.20 | 23.96 | 25.60 | 27.80 |
| Correlation coefficient | 0.96 | 0.97 | 0.97 | 0.99 |

### Stability Of Physicochemical Property Indicators

Periodic TLC, HPLC UV-visible and I.R. spectrophotometric monitoring of CLF in coevaporate of accelerated stability samples, showed no detectable change. The TLC showed a single spot (Rf-value= 0.34) and the HPLC showed a single peak (retention time 6.15 mins), corresponding to that of pure unprocessed sample. Similarly UV-visible scan exhibited maxima at 535 and 288 nm for both pure and that from coevaporate. Like-wise I.R. spectrum of CLF from coevaporate showed similar characteristic peaks as those of untreated sample, which was corroborated by the British Pharmacopoeia 1988.

### Accelerated Stability

Results of accelerated stability test are summarised in Table III a. Negligible amount of CLF was lost during the twelve-week period of study. The apparent decomposition kinetics at each incubation temperature could be described by either zero or first-order process. Calculated shelf-life ranged from 2 to 3 years.

Dissolution in acidic medium, conducted at each sampling time point, for all the three incubation temperatures (Table III b) showed over 85% release in two hours.

## DISCUSSION AND CONCLUSION

For water-insoluble or sparingly soluble drugs in solid dosage forms, dissolution may be a rate determining step in their absorption. A number of approaches have

## TABLE III

### Accelerated Stability Study Of Clofazimine In 1:10 Coevaporate

(a) Percentage of clofazimine remaining*

| Incubation time (weeks) | Incubation temperature | | | | | |
|---|---|---|---|---|---|---|
| | 25°C | | 37°C | | 50°C | |
| 2 | 98.82 | | 99.44 | | 100.31 | |
| 4 | 98.41 | | 99.37 | | 98.42 | |
| 6 | 99.67 | | 99.68 | | 99.61 | |
| 10 | 99.14 | | 99.52 | | 99.54 | |
| 12 | 99.00 | | 98.68 | | 99.08 | |
| Apparent decomposition rate constant | Zero-order (%/week) | First-order (1/week) | Zero-order (%/week) | First-order (1/week) | Zero-order (%/week) | First-order (1/week) |
| | 0.0353 | 0.0004 | 0.0537 | 0.0005 | 0.0437 | 0.0004 |
| Shelf-life (weeks) | 141.6 | 140.1 | 93.1 | 91.4 | 114.4 | 114.9 |

* Relative to the actual amount of CLF incorporated in coevaporate

(b) Dissolution in acidic medium**

| Incubation time (weeks) | Incubation temperature | | |
|---|---|---|---|
| | 25°C | 37°C | 50°C |
| 2 | 88.8 | 84.8 | 85.6 |
| 4 | 86.8 | 85.6 | 89.6 |
| 6 | 93.6 | 90.5 | 89.6 |
| 10 | 88.0 | 86.8 | 88.0 |
| 12 | 88.6 | 88.8 | 89.6 |

** %CLF released in 2 hours

been employed to solubilize such drugs and thereby increase systemic availability. These include solubilization in surfactant system[31], by cosolvency[32], through inclusion compounds[33], by drug derivitization[34] and through solid-dispersion[12, 13]. Solid dispersion, in which water-insoluble drugs are molecularly dispersed in water-soluble carriers, has been employed to improve the dissolution of a number of drugs, including indomethacin[35], sulfathiozole[36], griseofulvin[37], to name a few. In this study, we have attempted to improve dissolution of CLF by dispersing it in GAN-119.

Dissolution tests of the dispersion (coevaporate) in acidic medium (pH 1.2), buffer medium (pH7.5) and alkaline medium (pH10.0) demonstrated superior dissolution characteristics of CLF in coevaporate, relative to the pure, untreated compound and physical admixture (Fig.III a, b & c). The choice of dissolution medium was made to demonstrate the potential extreme solution pHs which the drug could be exposed to, in *in vitro* as well as under physiological conditions.

In all three solvents it was demonstrated that the extent of dissolution of CLF increased with polymer concentration in coevaporate.

With the physical admixtures 1:15 and 1:20, excepting acidic medium, the amount of CLF released was negligible and below the detection sensitivity of the analytical procedure. Lack of solubility of the admixtures in buffer and alkaline media may suggest that the polymer *per se* has little or no direct solvent effect, on the dissolution of CLF. The limited solubility of the two physical admixtures in acidic medium, may be due to the solvent effect of HCl since, protonation of CLF, which leads to its dissolution, is probably facilitated in this medium.

It would appear, therefore, that CLF and GAN-119 in coevaporate, constitute a unique association which readily dissolves in aqueous media. The nature of this association will be the subject of subsequent communication. It is hypothesized that the higher release rate from the coevaporate could be also due to increased protonation of CLF in the micro environment of the polymer. Polymer dissolution is initiated by hydrolysis to the free (maleic) acid-form, followed by its ionization. On ionization of the free acid, the high hydrogen ion concentration generated within the microenvironment of the polymer (which is less affected by stirring) could be expected to enhance protonation of CLF in intimate contact with polymer, leading to its rapid dissolution. It is worth noting that initial release rate of CLF from coevaporate in alkaline medium is the highest of the three media. This could be expected, since, hydrolysis of the polymer, which is the initiation step, will be favoured in alkaline medium. In acidic medium hydrolysis of anhydride of the polymer proceeds at a lesser rate than in alkaline medium. However, in the buffer medium, probably because of the constituent salt ($KH_2PO_4$) and the possibility of higher ionic strength than that prevailing in the acid or alkaline media, depressed solubility of polymer (due to "salting-out effect") also reduces the solubility of CLF. This salting-out phenomenon would be significant at low polymer concentration at which it cannot effectively compete with salt ions for water molecules. Thus CLF in 1:1 ratio coevaporate fails to dissolve in buffer medium, while it is soluble from the 1:5 ratio and above.

The release profiles (FIG.III) suggest that release of CLF from coevaporate is of zero-order. Table I represents results of linear regression analyses of % release *versus* time data, according to zero-order (Eq.8) or first-order (Eq.9). It is noted from the table that the intercept (which represents the % of CLF eventually released) as predicted by zero-order, is similar to experimental values and thus confirms zero-order release. It is also noted that at low polymer concentrations, the release pattern is kinetically indistinguishable between zero and first-order. This finding confirms the earlier report[38] which showed that drugs incorporated in polyvinylalkyl ether/maleic anhydride copolymer are released at a constant rate. This finding also offers GAN-119 as a potential agent for the preparation of sustained-release solid dosage form.

It is apparent from stability studies involving UV-visible and I.R. spectra and also TLC and HPLC that, CLF in coevaporate remained unchanged following preparation and storage. Accelerated stability studies at three selected incubation temperatures also indicate negligible loss of drug during storage, even at the elevated temperature of $50^0$C. This is significant in that the drug is largely used in tropical and subtropical areas of the world. Although apparent loss of CLF from coevaporate is negligible, the calculated shelf-life (2–3 years) is less than the reported time of 5 years for Lamprene®[8]. The difference may be attributed to sensitivities of the analytical methods employed in the two studies. It is also conceivable that any differences in incubation conditions and/or concentration monitoring periods could account for calculated shelf-lives.

It is reported that solid dispersion may suffer deterioration in dissolution properties after storage[39]. Periodic assessment of the extent of dissolution of 1:10 coevaporate in acidic medium, during the 12-week storage showed that over 85% of CLF is released within 120 minutes. This indicates that dissolution characteristics of CLF in coevaporate remained essentially the same during storage.

The systemic availability of CLF from coevaporate, relative to that from the conventional solid dosage-form in a suitable animal model, is currently being investigated.

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