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Effect of suppository bases on the release properties of a potent antimicrobial agent (C31 G)

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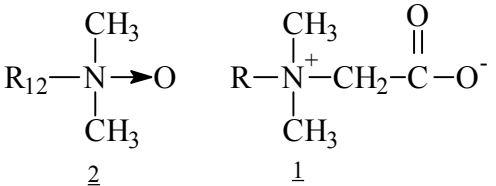
C31G is a specific formulation which contains equal molar concentrations of alkyi N-betaine and alkyl N.N-dimethylamine oxide. Vaginal suppositories containing 500 mg of C31G, this potent antimicrobial substance, were prepared by the fusion method in a variety of Suppocire® and Witepsol® bases with different melting points and hydroxyl values. *In vitro* release and diffusion characteristics of C31G from different suppository bases were investigated using two different systems. The release from suppositories was determined by using a system without a membrane and the diffusion rate of the released agent was determined through a semipermeable dialyzing tubing. Diffusion kinetics from suppositories were evaluated in terms of the apparent dialytic rate constants using the equation developed by Davis et al.
From the results of *in vitro* studies, Witepsol H 15 and Suppocire CM bases were selected as the most suitable ones for the formulations of C31G vaginal suppositories, since it is imperative for topical formulations to release the active substance in high proportions which are not absorbable by the mucosal membranes.

Freisetzung der antimikrobiellen Substanz C31G aus verschiedenen Suppositoriengrundlagen

Vaginalsuppositorien mit 500 mg C31G (Gemisch gleicher molekularer K-onzentrationen von Alkyl-N-betain und Alkyl-N.N-dimethylaminoxid) wurden mit Suppocire®- und Witepsol®-Suppositorienmassen unterschiedlicher Schmeiztemperaturen und Hydroxylgehalte hergestellt. Die Erfassung der Freisetzung von C31G erfolgte in einem System ohne Membran, die der Diffusionsrate mit einem semipermeablen Dialysierschlauch.
Fur Vaginalsuppositorien mit C31G erwiesen sich Witepsol H 15 und Suppocire CM als am geeignetsten.

1. Introduction

C31G is a unique mixture of synthetic amphoteric surface active compounds and contains equal molar concentrations of alkyl dimethyl glycine (alkyl N-betaine, coco betaine; **1**) and alkyl N,N-dimethylamine oxide (Cocoamine oxide; **2**).



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This mixture is patented in the USA [1] and is still under research. This composition exhibits germicidal and deodorizing properties and its use causes long term inhibition of body odor. C31G promotes the healing of infected and non-infected wounds in which the healing is histologically characterized by an increased rate of wound closure, increase in fibroblast infiltration and epithelization. Some studies have been reported on C31G concerning its healing effect on incised guinea pig wounds [2], evaluation of its effectiveness in dentistry by glycolytic tests and mouthwash formulations [3. 4], comparing its antipseudomonal activity against other topical agents [5]. evaluation of its surface active properties [6] and evaluation of its irritation on albino rabbit eyes [7]. Due to the stated properties of C31G. the goal of this work was to prepare topical vaginal suppository formulations which release the active substance in a short time but do not allow absorption of the active substance through the membranes of the vagina.

2. Investigations, results and discussion

The rate of drug release from dissolved or molten suppositories and the diffusion rate of dissolved drug molecules across the mucosal membranes are rate limiting factors of drug absorption in rectal or vaginal administration [8, 9]. As physicochemical properties of the base and the active substance determine the drug release, they are also known to be the main parameters affecting the absorption rate of drugs.
In our work, in order to investigate the effect of suppository bases on the release and diffusion characteristics of C31G, one hydrophilic (glycerinated gelatin) and six lipophilic (Witepsol® H15, Witepsol W31. Witepsol S55, Suppocire® AM, Suppocire CM and Massa Estarinum B) bases were tested. The amount of released and diffused C31G was found to be highest from glycerinated gelatin, therefore, water-miscible bases seemed to be more appropriate for systemic purposes than for topical application. Previously, it was also reported [10-12] that higher blood levels were obtained with water-soluble bases compared to lipophilic ones.
Synthetic suppository bases are mixtures of fatty acid esters with certain amounts of glycerides and their hydroxyl values represent the presence of mono and diglycerides, which also indicate the availability of free hydroxyl groups in the bases. In our formulations, higher release of C31G was predicted from bases with low hydroxyl values, as the chemical interaction between the active substance and the base was expected to be the least. In dissolution studies, at the end of 6 h, the highest percentages of released values were obtained from Suppocire CM, Witepsol H15 and Suppocire AM bases being 62.3%, 53.5% and 49.1%, respectively (Fig. 1). So, among the bases tested, Suppocire kinds seemed to release the active substance at higher levels. The melting range of Suppocire CM is 38-40 °C and Suppocire AM is 35-36.5 °C and the hy-

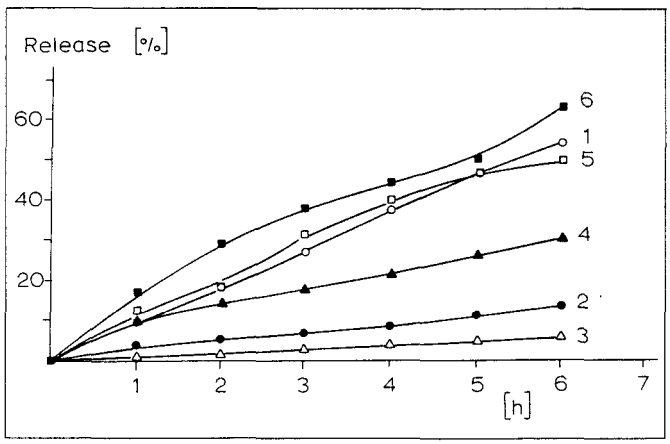

Fig. 1: *In vitro* release of C31G from various suppository bases. 1: Witepsol H15, 2: Witepsol W31, 3: Witepsol S55, 4: Massa Estarinum B, 5: Suppocire AM, 6: Suppocire CM

droxyl values of both bases are 6. The drug release from Suppocire CM was found to be higher than Suppocire AM. As these bases contain various kinds and amounts of mono and diglycerides. the active substance C31G might be incorporated with these mono and diglycerides. in different proportions which might have caused a difference in release pattern [13].

Drug partitioning is a function of the nature of base and it corresponds to the affinity of the drug towards bases. When there is low affinity between the drug and the base, the release rate of the substances having high solubility in aqueous media are expected to be high. The partitioning of the drug when using bases with high hydroxyl values appears to favour the lipid phase which is indicated by the lower rate of release. In our study, Witepsol H15. Suppocire AM and Suppocire CM bases were determined to have the lowest partitioning coefficients (0.45, 1.20, 1.57, respectively) (Table 1). As these three bases also have low hydroxyl values (15 < 6 < 6) when compared to others, it is possible to explain the high release percentage of the active substance C31G from the vaginal suppositories prepared using these bases.

Table 1: Evaluation of the partitioning of C31G between various suppository bases/pH 8 buffer and chloroform/pH 8 buffer (n = 3)

Base	Partition coefficient [K]
Witepsol H15	0.446
Witepsol S55	6.87
Witepsol W 31	1.73
Massa Estarinum B	2.69
Suppocire AM	1.20
Suppocire CM	1.57
Chloroform	6.83

C31G was found to be released less from Witepsol S55, Witepsol W31 and Massa Estarinum B bases. This might be due to the high hydroxyl (50-60, 25-35, 20-30 respectively) but low esterification values and also high partition coefficients they possess. Witepsol W31 also has the highest melting range of all the bases used (except for Suppocire CM). This might be an explanation for its low release. Witepsol S series comprises special grades and certain surface active agents which could act to enhance or retard drug release [13-15].

For topically applied formulations, the active substance is desired to be released from the bases in high proportions, but should not be absorbed from the membranes. Therefore we examined the diffusion behaviour of the active substance after it had been released from the base. The highest diffusion of the active substance was obtained from the water soluble base glycerinated gelatin which was beyond our scope. This base dissolves in aqueous media in a short time and docs not increase the viscosity of the dissolution medium, therefore in most cases the active substance is released and diffused faster than the lipophilic vehicles. In this work. the diffused amount was decreased from Suppocire AM (12.26%). Witepsol H15 (6.18%), Massa Estarinum B (4.61%) and Suppocire CM (4.56%) bases (Fig. 2). As Suppocire AM base has a low hydroxyl value and a low melting range (35-36.5 °C). release and diffusion of the active substance into the medium was determined to be fast.

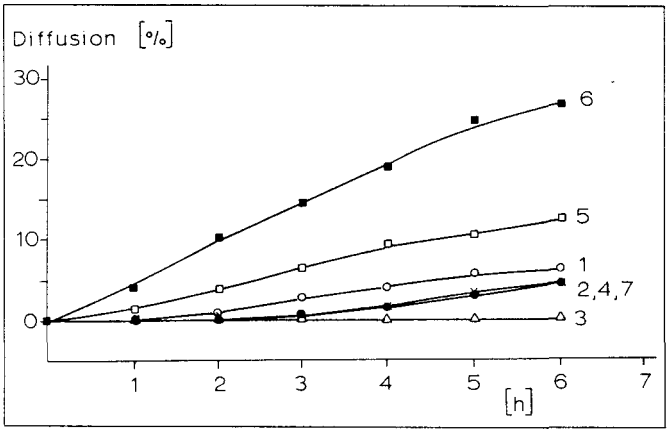

Fig.2: *In vitro* diffusion of C3 IG following the release from various suppository bases. I: Witepsol H 15, 2: Witepsol W 31, 3: Witepsol S55. 4: Massa Estarinum B, 5: Suppocire AM, 6: glycerinated gelatin, 7: Suppocire CM

The diffusion behaviour of the active substance from suppositories was also evaluated kinetically in terms of the apparent dialytic rate constants (Table 2).

Table 2: Calculated dialytic rate constants

Base	Dialvtic rate constant k [h ']
Witepsol H15	0.142
Massa Estarinum B	0.146*
Suppocire AM	0.238
Suppocire CM	0.142*
Glycerinated gelatin	0.606

* Calculated after 2 h

The equation developed by Davis et al. [16] was used for the calculation of the apparent dialytic rate constant: where V, is the volume of the test medium in the dialysis bag, V,, the volume of the test medium outside the dialysis bag, A,, the amount of drug dialyzed, A, the total amount of drug in the test sample, t the time, and k the apparent dialytic rate constant.

$$\log\left[V_0A_t - (V_0+V_i)A_0\right] = -\left[\frac{V_0+V_i}{2.3V_iV_0}\right]kt + \log(V_0A_t) \qquad (1)$$

When the term log $[V_0A - (V_0+V_i)\ A_0]$ representing the amount of drug remaining in the dialysis bag is plotted c.s-time, a straight line is obtained. The apparent dialytic rate constant k can be evaluated as follows:

$$k = \frac{-(\text{slope})\ (2.3)\ (V_iV_0)}{Vi + V_0} \qquad (2)$$

In the Tables 1 and 2 the values of apparent dialytic rate constant k, and the partition coefficient values K of each investigated suppository base were given. Data showed that the drug release was fastest from suppositories prepared with glycerinated gelatin and Suppocire AM. Witepsol H 15 with the lowest partition coefficient released the drug at a slower rate, therefore, a general correlation between K and k values could not be drawn.

Before the formulation attempt and *in vitro* testing in a bacteriological medium, a microbiological study of C31G was accomplished in which the minimum inhibitory concentrations (MIC) and the effective period for cidal concentrations (MCC) of the C31G mixture were determined on 13 microoorganisms, a series of common bacteria and fungi that are known to cause vaginitis by the microtiter dilution procedure [17]. Also, in this previous work, the antimicrobial efficiency of this compound was evaluated on 105 culture samples which were obtained from patients of Turkish population having vaginal infection. As a result, *E. coli* (45.45%) and *S. aureus* (19.69%) were found to be the most frequently observed microorganisms in these samples and C31G was determined to be active on microorganisms obtained from the vagina at very low concentrations in a short time. The MIC of C31G on *S. aurens* was determined to be 0.006% actives and on *E. coli* 0.2% actives.

After these microbiological findings, an attempt has been made to evaluate the *in vitro* release properties of the two formulations of C31G prepared with Witepsol H 15 (formulation A) and Suppocire CM (formulation B) bases in a bacteriological

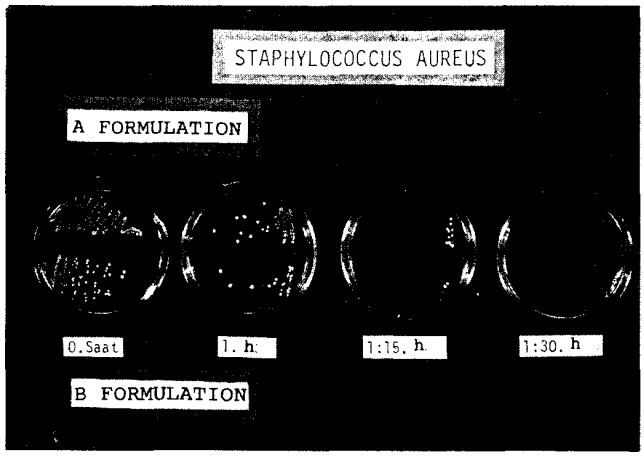

Fig. 3: Evaluation of the antimicrobial efficiency of formulations A and B on S. *aurrus* as a function of time

release medium using the procedure explained in the experimental section. For the 5. *aureus* strain, in the control plate, the growth of microorganisms were obvious (Fig. 3).
The microorganism growth was evaluated continuously in 15 min intervals and the reduction in microorganism colonies was clearly observed. When the 1 h sample was examined closely, this reduction became so clear, indicating the microbiological efficiency of C31G after being released from the suppositories. At the end of 1.5 h. there was no growth of microorganism on the plates where formulations A and B were applied, indicating that this was an adequate period for the active substance to be 100% effective on *S. aureus* strain. The results obtained from formulations A and B against the *E. coli* strain are shown in Fig. 4. Formulations A and B were 100% effective against the *E. coli* strain in 4.5 h.
As a result of this present work, Witepsol H 15 and Suppocire CM suppository bases from which the highest release and the lowest diffusion rates were obtained were concluded to be the most suitable bases for the vaginal application ofC31G, a potent antimicrobial agent, for treatment of vaginitis. Microbiological experiments concerning *in vitro* release also revealed that after being released from suppositories, C31G showed antimicrobial activity against the tested microorganisms. After controlling the vaginal irritancy of C31G, these findings should also be confirmed by *in vivo* experiments.

3. Experimental

3.1. Materials and instruments

C31G (29.5% actives. Hunlerdon Pharmaceuticals. batch No: 001 USA), Witepsol H 15.Witepsol S55, Witepsol W31.Massa Estarinum B (Dynamit Nobel).Suppocire AM. Suppocire CM (Gatlefosscl. gelalin. glycerol (Merck). dialyzing tubing (Spectropor 2. molecular cut off: 12000- 14000. cylinder diameter: 28.6 mm). Lclheen agar (Difco). Mueller-Hinlon broth (Oxoid). All materials were used as received from the manufacturer or distributor with no further purification.
Ultrasonic bath (Bransonic 220). refrigerated centrifuge (MLW. model K 24). UV spectropholometer (Hitachi, model 220)

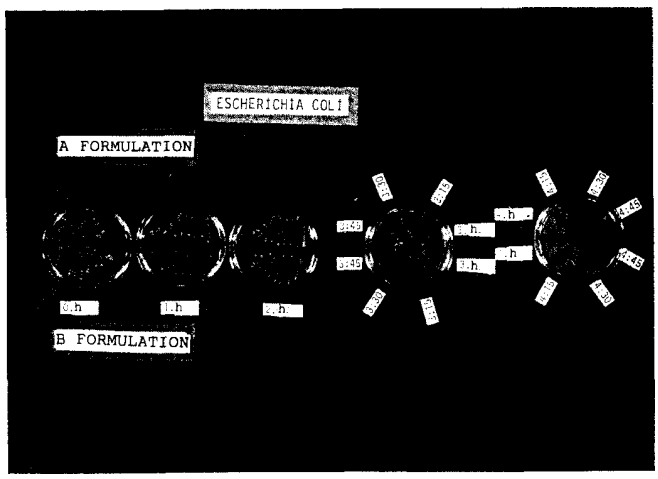

Fig. 4: Evaluation of the antimicrobial efficiency of formulations A and B on E. *coli* as a function of time

3.2. Methods

3.2.1. Preparation of suppositories

Vaginal suppositories containing 500 ing of C31G were prepared by the fusion method using a water-soluble base. givcerinaled gelatin and six oleagenous bases—Witepsol H 15, Witepsol S55, Witepsol W31. Suppocire AM. Suppocire CM. Massa Estarinum B. After calibration of the molds, a sufficient amount of C31G for 12 suppositories was incorporated by geometric dilution into the just molten bases.After thorough mixing, the mass was poured into unlubricated molds and allowed to solidify at room temperature. Blank suppositories containing no active substance were prepared to determine the absorbance of each base. Suppositories were packed in tightly closed containers and stored in a refrigerator. They were left at room temperature for 12 h before carrying out each evaluation.

3.2.2. Evaluation of suppositories

3.2.2.1. Assay procedure

The ampholeric surface active components of C31G form a precipitate at pH 1 when reacted with ammonium Reineckate. For quantitative determination, the precipitated complex is dissolved in acetone and the color is determined at 525 nm [18, 19].

3.2.2.2. Determination of partition coefficient

Phosphate buffer solution (10 ml. pH 8) containing 5 mg/ml of active substance. was agitated with each of the lipophilic suppository bases (3 g) in screw-capped tubes. Tubes were placed in a mechanical shaker with a thermostated water bath at 38 °C. At this temperature, all of the bases melted. Tubes were agitated at 50 cpm for 6 h which was adequate to attain equilibrium. After equilibrium. the tubes were placed in the refrigerator in order to separate the lipophilic phase by freezing.Then. the aqueous phase was filtered and the active substance assayed spectrophotometrically. The partition coefficient of C31G between the lipid and water phases was calculated according to Ritschel [20]. Data given in Table 1 are the results of three parallel experiments.

3.2.2.3. *In vitro* determination of the release of C31G

The release system was composed of a glass beaker containing 20 ml phosphate buffer (pH 8) in which a suppository was placed and stirred with a magnet at 100 rpm at 37 ± 0.5 °C. At constant time intervals (1, 2, 3, 4, 5 and 6 h) 2 ml samples were removed from the release medium and assayed by UV spectrophotometry [19] to determine the release characteristics.

3.2.2.4. *In ritro* determination of the diffusion of C31G

The prepared suppositories were tested for *in vitro* diffusion, adopting the method of Plaxco et al. [15]. Dialyzing bags were prepared from dialyzing cellophane tubing (Spectropor 2) tied with cotton thread and soaked overnight in the phosphate buffer solution (pH 8). After rinsing the bags twice, 20 ml of phosphate buffer solution (pH 8), one suppository and glass beads with 0.3 cm diameter, in order to prevent the precipitation ofC31G due to the low viscosity of some bases used. were placed in each bag and suspended in a glass diffusion cell containing 100ml of the phosphate buffer solution (Fig, 5). The diffusion system was placed in a constant temperature water bath at 37 ± 0.5 °C and agitated with a magnetic stirrer at 200 rpm. At constant time intervals, samples (5 ml) were removed from the diffusion cell and assayed to obtain a diffusion profile. Phosphate buffer (5 ml) were added to the medium to compensate for

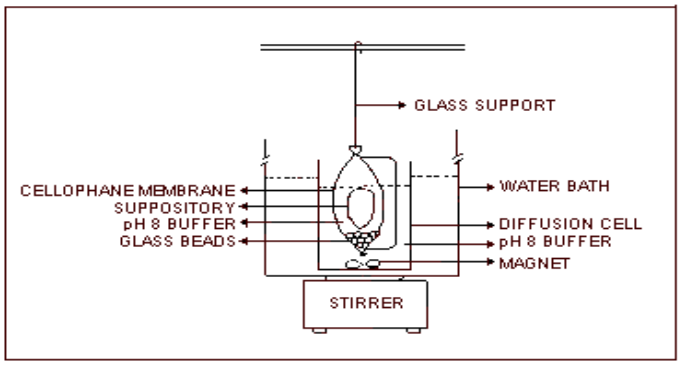

Fig.5. *In vitro* diffusion system

Sampling. The absorbances were measured colorimetrically at 525 nm and the concentrations were obtained from the standard curve.

3.2.2.5. Microbiological studies

Tile antimicrobial clficiency of two formulations which were concluded to be tlie most appropriate (prepared with Witcpsol H15 and Suppocire CM bases. formulation A and B respectively) for the aim of this work were investinated on *Escherichia coli* and *Staphylococcus* strains[1]—the most frequently observed microorganisms, in culture sampies of Turkish women [20] — as a function of time.

Culture samples (10 ml) of microorganisms (5.10^5 CFU/ml) were added to 10 ml bacteriological medium (Mueller Hinton broth), then a suppository was placed and incubated at 37 °C.

Samples were taken at 15 min intervals with a sterile loop and exposed to Letheen Agar. After incubating the plates for 18 h at 37 ^{0}C. microorganism growth was evaluated.

[1] Isolates of Microbiology Department of Faculty of Medicine of Hacettepe University

References

1 Micheals. E. B.: US patent 4, 107, 328 (1978)
2 Micheals. E. B.: Hahn. E. C.: Kenyon. A. J.: Am. J. Vet. Res. **44**, 1378 (1983)
3 Corner. A. M.: Dolan, M. M., Malamud. M. M.: Yankell, S. L.: J. Dent, Res. **65,** 274 (1986)
4 Malamud. D.: Corner. A. M.; Dolan. M. M.; Hammond, B. F.: ibid. **65.** 275 (1986)
5 Stieritz, D. D.: Bondi. A.: McDermott. D.: Micheals, E. B.: J. Antimicrob. Chemother. **9**, 133 (1982)
6 Unlu. N.. Calis, S.: Sumnu. M.: Hincal. A. A.: Boll. Chim. Farm. **130,** 234 (1991)
7. Unlu, N.: Calis, S.: Irkec, M., Aksoy, F.: Orhan, M.: Sumnu, M.: Hincal, A. A.: STP Pharma Sci. (Special Issue on Ocular Administration) **2**, 45 (1992)
8. de Blaey, C. J.: Poiderman, J.: Rationales in the Design of Rectal and Vaginal Delivery From of Drugs, p. 237; in: Ariens. E.J.(Ed.) 1: Drug Design. Vol. 9. Academic Press. New York 1980
9. Casahoursat. L.: S.T.P. Pharma **4**, 572 (1988)
10. Vidras, N. J.: Vincent, E. R.: Bohidor, N. R.: Plakogiannis, F. M.: J. Pharm. Sci. **71**, 945 (1982)
11. Kakemi, K.: Arita, T.: Muranishi, S.: Chem. Pharm. Bull. **13**, 976 (1965)
12. Kawashima, S.: Nakazawa, H.: Fujiwara, H.: ibid. **36**, 1475 (1986)
13. Becirevic, C, M.: Petncic, V.: Kellay, N,: Pharmazie **39.** 828 (1984)
14. Othman, S.: Muti, H.: Drug Dev. Ind. Pharm. **12,** 1813 (1986)
15. Plaxco, J. M.: Free, C. B.: Rowland, C, R.: J. Pharm. Sci. **56**, 809 (1967)
16. Davis, R. E.: Hartman, C, W.: Eincher, J. H.: ibid. **60**, 429 (1971)
17. Calis, S.: Yulug, N.: Sumnu, M.: Ayhan, A.: Hincal, A. A.: Boll. Chim. Farm, **131**, 335 (1992)
18. Nishida, M.: Ryuichi, M.: Walanabe, V,: Usui, K.: Kanazawa Daigaku Kogakubu Kiyo **4**, 189 (1966)
19. Calis, S,: Calis, U.: Sumnu, M.: Hincal, A. A.: Doga-Tr. J. Pharm. **1**, 97 (1991)
20. Ritschel, W. A.: Handbook of Basic Pharmacokinetics. 3rd Ed., p. 71. Drug Intelligence Publications. 1986

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