DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 10(4), 685-697 (1984)

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/21/12
For personal use only.

EVALUATION OF THE BIOLOGICAL ACTIVITY OF SOME MEDICATED SOLIDIFIED SODIUM STEARATE-BASED STICKS (SSSS).

Medhat Abdel-Hamid*, Ali A.Kassem**, Amir G.Mattha*** and Gaber K.El-Khatib**

*Faculty of Medicine, El-Azhar University, **Faculty of Pharmacy, Cairo University, ***Laboratory of Pharmaceutical Sciences, National Research Center, Dokki, Cairo, Egypt.

ABSTRACT.

The biological activity of Panthenol, Chlorphenesin and Lignocaine medicated solidified sodium stearate-based sticks (SSSS) was evaluated in an assessment of the suitability of this dosage form for topical treatment.

Panthenol sticks were evaluated as to their healing promoting activity by producing sterile ulcerated circular areas on the shaved hind limbs of male albino rats, applying a definite amount of the drug to them and observing their rate of healing.

Chlorphenesin sticks were evaluated as to their antifungal and antibacterial effects on two fungi, Trichophyton mentagrophytes and Candida albicans as well as on Staphylococci. This necessitated a prior screening of the base components, viz. alcohol and humectants individually for these effects on culture media using Sabouraud's glucose-agar and blood-agar; no effect was noticed. The activity of the sticks was then studied by infecting about 5cm^2 of the scratched skin of the four limbs of guinea pigs with the microorganisms, applying a definite amount of the drug

Copyright © 1984 by Marcel Dekker, Inc. 0363-9045/84/1004-0685$3.50/0

RIGHTSLINK

to them three times daily and observing the degree of healing obtained after three days treatment.

Lignocaine sticks were evaluated as to their local anaesthetic activity by application to the tail of male albino rats and the observation of the duration of their anaesthetic effect using the Hardy-Wolff-Goodell pain threshold apparatus modified by Tye.

The SSSS dosage form proved to be as efficient as proven respective ointment brands containing the investigated medicaments in equal concentration.

INTRODUCTION.

Previous publications have considered the influence of three topically active drugs,Panthenol,Chlorphenesin and Lignocaine,on the physical characteristics (1)and rheological properties (2) of some solidified sodium stearate-based sticks(SSSS)and the stability of these drugs in such a dosage form (3).The present work aims at evaluating the biological activity of some medicated sticks in an assessment of the suitability of this dosage form for topical treatment.For this purpose,the wound healing activity of Panthenol sticks (4),the antifungal and antibacterial activity of Chlorphenesin sticks (5) and the local anaesthetic activity of Lignocaine sticks (6,7),were evaluated using laboratory animals and simple well established techniques,comparing their effect to that of proven respective ointment brands containing the investigated medicaments in equal concentration.

MATERIALS.

-Plates of Sabouraud's Glucose-Agar.

-Plates of Blood Agar.

-D-Panthenol,from Hoffmann-Laroche,Switzerland.

-Chlorphenesin (B.P.),from B.D.H.,London,England.

-Lignocaine(Lidocaine)(U.S.P.),from Dotto-Bonapace,Milano, Italy.

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/21/12
For personal use only.

RIGHTSLINK

-Samples of medicated Panthenol,Chlorphenesin and Lignocaine SSSS containing 15% glycerol,propylene glycol (PG) or Polyethylene glycol 600 (PEG 600) and their corresponding medicament free bases,prepared as mentioned in a previous communication (1).

-Terramycin drops,containing 100 mg/ml of oxytetracycline HCl, Pfizer,Egypt.

-Panthenol 5% ointment and Lignocaine 2% ointment,both from the Nile Company for Pharmaceuticals and Chemical Industries,Egypt.

-Fungicil ointment,containing Chlorphenesin 1%,Chemical Industries Development Company,Egypt.

APPARATUS.

-Electrically heated,thermostatically controlled hot-air oven,local production.

-Microscope,binocular,Zeiss,G.D.R.

-Hardy-Wolff-Goodell pain threshold apparatus modified by Tye,consisting mainly of a 500 Watts lamp.

ORGANISMS.

-Fungi:Trichophyton mentagrophytes,
Candida albicans.

-Bacteria:Staphylococci.

Animals.

-Male albino rats:150-250g each.

-Guinea pigs:300-500g each.

PROCEDURES.

Evaluation of the Biological Activity of Panthenol Sticks:Panthenol sticks containing 15% glycerol were evaluated as to their healing promoting activity (4)using male albino rats,as follows:

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/21/12
For personal use only.

RIGHTSLINK

-The animals were divided into seven groups,each of 8 rats, in separate cages,fed on milk,carrots and tap water containing 25mg Oxytetracycline hydrochloride per 100ml to prevent secondary infection of the induced burn ulcers.

-The area of the skin of their hind limbs was shaved and exposed for burning with concentrated hydrochloric acid in order to produce sterile ulcerated circular areas of about 2cm diameter each.

-The investigated sticks were applied twice daily in the different tested concentrations (1,3,5,7 and 10%)by rubbing them over the ulcerated areas to yield a total quantity of 0.5g on the area each time,using five groups of animals.

-The rate of healing was noticed and compared to that in a sixth group receiving non medicated sticks.

-A seventh group was treated with Panthenol ointment (5%), instead of Panthenol sticks.

Evaluation of the Biological Activity of Chlorphenesin Sticks: Chlorphenesin sticks containing 15% glycerol or PG were evaluated as to their antifungal and antibacterial activity (5)using two fungi,Trichophyton mentagrophytes and Candida albicans,as well as Staphylococci.This necessitated a prior screening of the base components,viz.alcohol and humectants liable to be present in the stick,individually,for this activity,in absence and presence of Chlorphenesin,or culture media using Sabouraud's glucose-agar and blood-agar.The activity of the sticks was then studied by infecting guinea pigs with the same organisms and observing the degree of healing of the infected areas (5).

Screening of the Antifungal and Antibacterial effects of Chlorphenesin in Solution:The Sabouraud's glucose-agar method (5) was used for screening the antifungal activity.Different sets of Sabouraud's media,each composed of two plates,one for Trichophyphyton mentagrophytes and the other for Candida albicans,were prepared and specified as follows:

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/21/12
For personal use only.

RIGHTSLINK

-For screening the antifungal activity of 1% Chlorphenesin in glycerol,PG,PEG 600 and alcohol 70%,one set of plates for each type of solution was used.

-For screening the possibility of any antifungal activity of the above mentioned solvents,one set of plates for each solvent was used.

For screening the antibacterial activity of the above mentioned solutions and solvents,Staphylococci cultures in blood agar plates were used,one for each solution or solvent.

Sabouraud's glucose-agar was prepared and cooled to 45^{o};100ml medium was mixed with 1ml spore suspension and poured into sterile plates.After solidification,four bores (0.7cm diameter)were made in each plate.These were filled with the various respective investigated solutions and solvents.The plates were incubated at 28^{o}.In each case,the diameter of the inhibition zone reported was the mean of four measurements.The inhibition zone diameters of Candida albicans were measured after 24 hours and those of Trichophyton after 96 hours.

Analogous plates containing blood-agar cultures of Staphylococci,were measured after 48 hours.

Screening the Antifungal and Antibacterial Effects of Chlorphenesin Sticks:Panthenol sticks containing 15% glycerol or PG were evaluated as to their antifungal and antibacterial activity (5) as mentioned below.PEG 600 -formulated sticks were eliminated since 1% Chlorphenesin solutions in PEG 600 and Glycerol showed almost similar behaviour(fig.2).The experiment was conducted as follows:

-Four groups of guinea pigs,each of 6 animals,were infected by scratching the skin of their four limbs by a fine needle contaminated with various active cultured organisms or fungi.

-The tested medicated stick was rubbed three times daily over the infected area (about $5cm^{2}$)for three successive days.The rubbed quantity was adjusted to be 0.5g in each application.The degree of healing was observed during this period,taking into consideration that each pair of limbs was specified to the medicament in a

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/21/12
For personal use only.

RIGHTSLINK

certain base,on one limb,with its corresponding base,on the other. According to the type of infection,the animals were classified as follows:

-Group 1,infected with Trichophyton mentagrophytes.

-Group 2,infected with Candida albicans.

-Group 3,infected with Staphylococci.

-Group 4,used to compare the antifungal effect of Chlorphenesin sticks,with that of the drug in a hydrophilic ointment base (Fungicil),since the antimycotic activity of Chlorphenesin was reported to be maximal in this type of bases (8).

Evaluation of the Biological Activity of Lignocaine sticks:Lignocaine sticks were evaluated as to their local anaesthetic activity on the basis of the response of albino rats to a heat stimulus applied to their tail (6,7).The apparatus employed was the Hardy-Wolff-Goodell pain threshold apparatus modified by Tye (9) and consisting mainly of a 500 Watts lamp,the light beam focused on an aperture by a condensing lens,was electronically controlled in intensity and time by suitable devices.The pain threshold of the animals was determined as follows:the tail of the rat was immersed in a 1% aqueous solution of Lignocaine hydrochloride for 30 minutes then taken out and the excess solution removed by gentle dabbing with cotton.The tail was then coated with India ink.When the ink had dried,the animal's tail was subjected to 400 millicalories in the Hardy-Wolff-Goodell pain threshold apparatus at one minute intervals until a positive reaction to the stimulus was noted.A slight twitch or quiver was regarded as a negative response,a jerk or removal of the tail as a positive one.The pain threshold of each animal was checked before each experiment.

The medicament (as 15% glycerol-formulated stick or as ointment)was applied to the rat's tail,allowed to act for one hour and removed.The tail was then prepared with India ink and tested in the pain threshold apparatus.Each test was conducted on a group of 6 animals.

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/21/12
For personal use only.

RIGHTSLINK

RESULTS AND DISCUSSION.

Evaluation of the Biological Activity of Panthenol Sticks:Figure 1 shows that the mean times elapsed after the application of Panthenol sticks containing 5-10% Panthenol and Panthenol (5%)ointment, in hydrophilic base are practically the same.Below 5% concentration, the activity of Panthenol is seen to be reduced.Blank sticks containing no Panthenol show the longest time.

Screening of the Antifungal and Antibacterial Effects of Chlorphenesin in solution:Whereas 70% ethanol,glycerol,PG and PEG 600 showed no inhibition zone at all in absence of Chlorphenesin,1% Chlorphenesin solutions in these solvents had a positive action on the three investigated microorganisms (fig.2);this action was slightly weaker in presence of ethanol than with the humectants which showed practically equivalent effects.

Screening the Antifungal and Antibacterial Effects of Chlorphenesin Sticks:Albino rats infected with the test fungi had zones of infection with diameters ranging from 2.5 to 3.5 cm at the beginning of the experiment;the application of 1% Chlorphenesin sticks,whether glycerol or PG-formulated,resulted in the disappearence of the infection at the end of the treatment;the same happened with the ointment preparation;on the other hand,blank sticks,containing no medicament,had no effect and the diameters of the infected zones to which these sticks were applied ranged between 2.7 and 3.8cm at the end of the experiment.

As regards the influence of Chlorphenesin sticks (1%) on the Staphylococci infections induced in the test animals,table 1 shows that the diameter of the area infected by Staphylococci ranged from 2.9 to 3.3cm at the beginning of the experiment;the application of the medicated sticks,whether glycerol or PG-formulated,to the infected areas resulted in complete cure in 60% of the cases at the end of the treatment ,while mild residual infected areas remained in 40% of these animals.

Evaluation of the Biological Activity of Lignocaine Sticks:Table 2 shows that the duration of anaesthesia after the application of

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/21/12
For personal use only.

RIGHTSLINK

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/21/12
For personal use only.

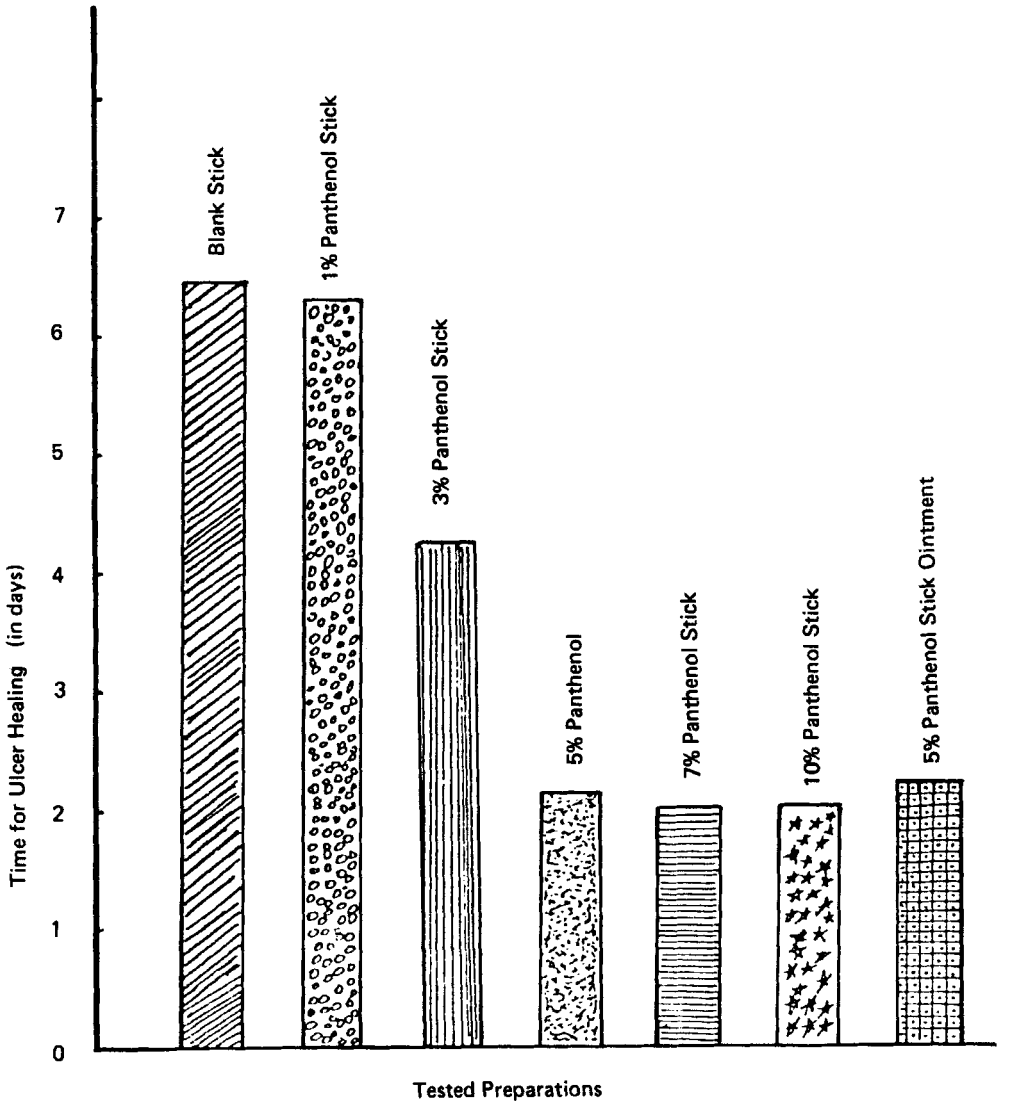

FIGURE 1

RIGHTSLINK

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/21/12
For personal use only.

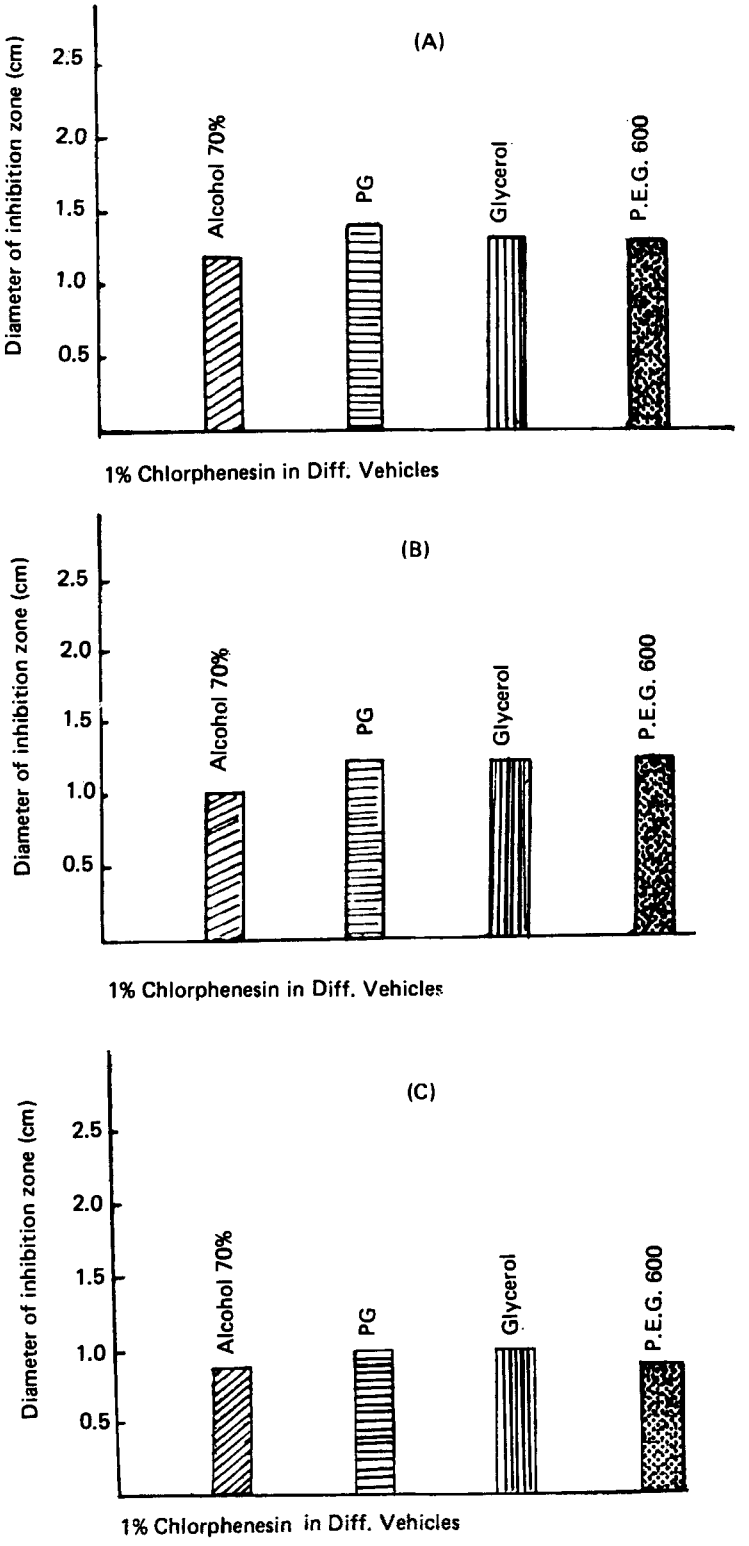

FIGURE 2

RIGHTSLINK

TABLE 1
Screening of the Effect of 1% Chlorphenesin Sticks (Glycerol or PG-formulated) on Staphylococci.

Animal Number	Diam. of Infected Zone (cm)	Medicated Stick, G-F* LFL+	Blank Stick, G-F RFL++	Medicated Stick, PG-F** LHL+++	Blank Stick, PG-F RHL++++
1	RFL 3.1 LFL 3.1 RHL 3.2 LHL 3.2	Cured	3.5	Cured	3.4
2	RFL 3.3 LFL 3.3 RHL 3.2 LHL 3.3	0.5cm left	3.6	0.5cm left	3.4
3	RFL 3.0 LFL 3.0 RHL 3.0 LHL 3.1	Cured	3.5	Cured	3.2
4	RFL 2.9 LFL 3.0 RHL 3.1 LHL 3.0	0.4cm left	3.4	0.5cm left	3.2
5	RFL 3.2 LFL 3.2 RHL 3.1 LHL 3.2	Cured	3.5	Cured	3.2
6	RFL 3.3 LFL 3.1 RHL 2.9 LHL 3.2	Cured	3.6	0.3cm left	3.1

*G-F: Glycerol-formulated **PG-F: PG-formulated
+RFL: Right Forelimb ++LFL: Left Forelimb
+++RHL: Right Hind Limb ++++LHL: Left Hind Limb.

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/21/12
For personal use only.

RIGHTSLINK

TABLE 2
Effect of Lignocaine(2%)Sticks on the Mean Duration of Local Surface anaesthesia in Male Albino Rat's Tail.

	Serial No.of Test Animal						Mean Durn. (min)
	1	2	3	4	5	6	
Duration of Anaesthesia After Application of Blank Stick Base	0	0	0	0	0	0	0
Duration of Anaesthesia After Application of 2% Lignocaine Sticks	15.4	11.6	16.0	16.0	15.3	13.3	14.6

TABLE 3
Effect of Lignocaine (2%)Ointment on the Mean Duration of Local Surface Anaesthesia in Male Albino Rat's Tail.

	Serial No.of Test Animal						Mean Durn. (min)
	1	2	3	4	5	6	
Duration of Anaesthesia After Application of Blank Ointment Base	0	0	0	0	0	0	0
Duration of Anaesthesia After Application of 2% Lignocaine Ointment	16.0	14.0	16.0	12.0	12.0	14.0	14.0

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/21/12
For personal use only.

RIGHTSLINK

blank sticks was zero,while after application of 2% Lignocaine sticks,the mean duration of local surface anaesthesia was of 14.6 minutes.Lignocaine ointment gave a mean duration of local surface anaesthesia of 14minutes,while its corresponding base failed to give any effect (table 3).

CONCLUSIONS.

-The healing promoting activity of Panthenol sticks containing 5-10% medicament is equivalent to that of the Panthenol ointment brand.

-Sticks having a Panthenol content below 5% show a lower healing promoting activity than those with a higher medicament concentration.

- 70% ethanol,glycerol,propylene glycol and PEG 600 have no influence on the tested fungi and Staphylococci under the test conditions;1% Chlorphenesin solutions in these solvents have a positive action on these microorganisms ;however,the action of 70% ethanol solutions is slightly weaker.

-The antifungal effect of Chlorphenesin sticks is equivalent to that of the Chlorphenesin ointment brand.

-The local surface anaesthetic effect of Lignocaine sticks is equivalent to that of the Lignocaine ointment brand.

-Blank SSSS show none of the above tested biological activities.

-The results confirm the suitability of SSSS as a topical dosage form,at least in the investigated cases.

REFERENCES.

1.A.A.Kassem,A.G.Mattha and G.K.El-Khatib,Drug Development and Industrial Pharmacy

2.A.G.Mattha,A.A.Kassem and G.K.El-Khatib,Drug Development and Industrial Pharmacy

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/21/12
For personal use only.

RIGHTSLINK

3.A.A.Kassem,A.G.Mattha and G.K.El-Khatib,Drug Development and Industrial Pharmacy

4.G.D.Novell,Physiol.Reviews,33,525(1953).

5.G.H.Smith and I.Sarkany,"Fungus Diseases and their Treatment", A.Churchill Ltd.,London,1964,p.31.

6.W.L.Mack and J.W.Nelson,J.Am.Pharm.Assoc.Sci.Ed.,42,101(1953).

7.W.L.Mack and J.W.Nelson,J.Am.Pharm.Assoc.Sci.Ed.,42,103(1953).

8.E.E.Galal,A.El-Shahed,H.El-Baghdady,A.El-Shami,M.A.Moustafa, A.Gobba,M.Refaei and A.A.Kassem,J.Drug Res.Egypt,5,205(1973).

9.A.Tye and B.V.Christensen,J.Am.Pharm.Assoc.Sci.Ed.,40,404(1951).

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/21/12
For personal use only.

RIGHTSLINK