Assessment of Precorneal Residence of Timolol maleate by HPTLC Technique

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INTRODUCTION

OCULAR DISORDERS

Eyes become sick due to several reasons such as

- Improper working conditions
- Poor nutrition
- Poor ventilation and illumination
- Excessive use of vibrating machinery, chemicals and
- Injury to head or eyes

The conventional ocular delivery systems (CODS) are

- Solutions – eye drops
- Ointments
- Injections and
- Eye irrigation solutions.

CODS - Drawbacks

Poor ocular bioavailability due to

- Solution drainage
- Lacrimation
- Tear dilution
- Tear turnover (about 16%) and
- Conjunctival absorption.
- Binding of drug to protein

only a small amount (1-3%) actually penetrates the cornea and reaches the intraocular tissues

OCULAR DRUG DELIVERY SYSTEMS -ODDS

Objectives:

- To improve ocular drug bioavailability from 1-3% to 15-20%
- To decrease the frequency of administration
- To minimize the local and systemic side effects due to excessive nasolachrymal drainage and higher dose intake
- cost effective

ODDS - APPROACHES

- Aqueous suspensions
- Viscosity imparting agents
- Prodrugs and ion pairs
- Ointments
- Liposomes
- Nanoparticles
- Gel
- *Insitu*-gelling systems
- Non-erodible insert ocusert
- Erodible insert

An ideal ocular delivery approach must

- Improve the fraction of dose absorbed
- Maintain desired concentration of drug in various segments of the eye and
- Must increase the corneal drug residence

ODDS – **OUR APPROACH**

- Formulation of sodium alginate - based
  - Ocular films
  - Surface cross linked ocular films (SC-Films)
  - Micro spheres-dispersed ocular films (MS-Films)
  - Accurate-Dose-Detachable-Sterile-Inserts (ADDSerts)

SELECTION OF DRUG - TIMOLOL MALEATE

- Timolol is a promising medication for the treatment of glaucoma
- More effective in lowering intraocular pressure
- Better tolerated.

PROFILE OF TIMOLOL MALEATE

- Generic name : Timolol Maleate
- IUPAC name : (s)-1-tert-butylamino-3-(4 –morpholino -1,2,5,-thiadazol-3 yl oxy) propan-2 -ol maleate
- Molecular formula : $C_{13}H_{24}N_4O_3S$, $C_4H_4O_4$
- Molecular weight : 432.5
- Melting point : 201.5 – 202.5º C
- Official status : IP´96, BP, USP.

FORMULATION OF TIMOLOL MALEATE OCULAR FILMS

- Matrix type ocular films of timolol maleate :
  - ✓ prepared by moulding technique
  - ✓ A glass mould of 10 cm length, 5 cm width and 1.5 cm height was fabricated
  - ✓22.5 mg of timolol maleate + 17 ml of water + plasticizer ---- This is transferred in to sodium alginate solution and stirred for 2 h - poured into glass mould -- drying at 50º C for 6 h -- After drying, the film was removed and cut into pieces of 0.5 cm^2 size.

FORMULATION OF SURFACE CROSSLINKED (SC) FILMS

- Calcium chloride was used as surface cross linking agent for sodium alginate films, in which calcium reacts with sodium alginate to form insoluble calcium alginate on the surface

- This reduces the hydrophilicity of the film and release from polymeric matrix

FORMULATION OF MICROSPHERE – DISPERSED FILMS

- chemical crosslinking method - 10%w/v glutaraldehyde saturated toluene was used as crosslinking agent.
- 2 ml of 25 mg drug containing aqueous solution (25% w/v) was added to a mixture of 25 ml of linseed oil and 5 ml of toluene ---- desired size
- 2ml of Glutaraldehyde saturated toluene (GST) was added
- The suspension of microspheres was washed three times with 5 ml volumes of toluene
- The microspheres subjected to ***vitro*** drug release
- Selected batch was dispersed in sodium alginate and

MS- films were formulated by moulding technique

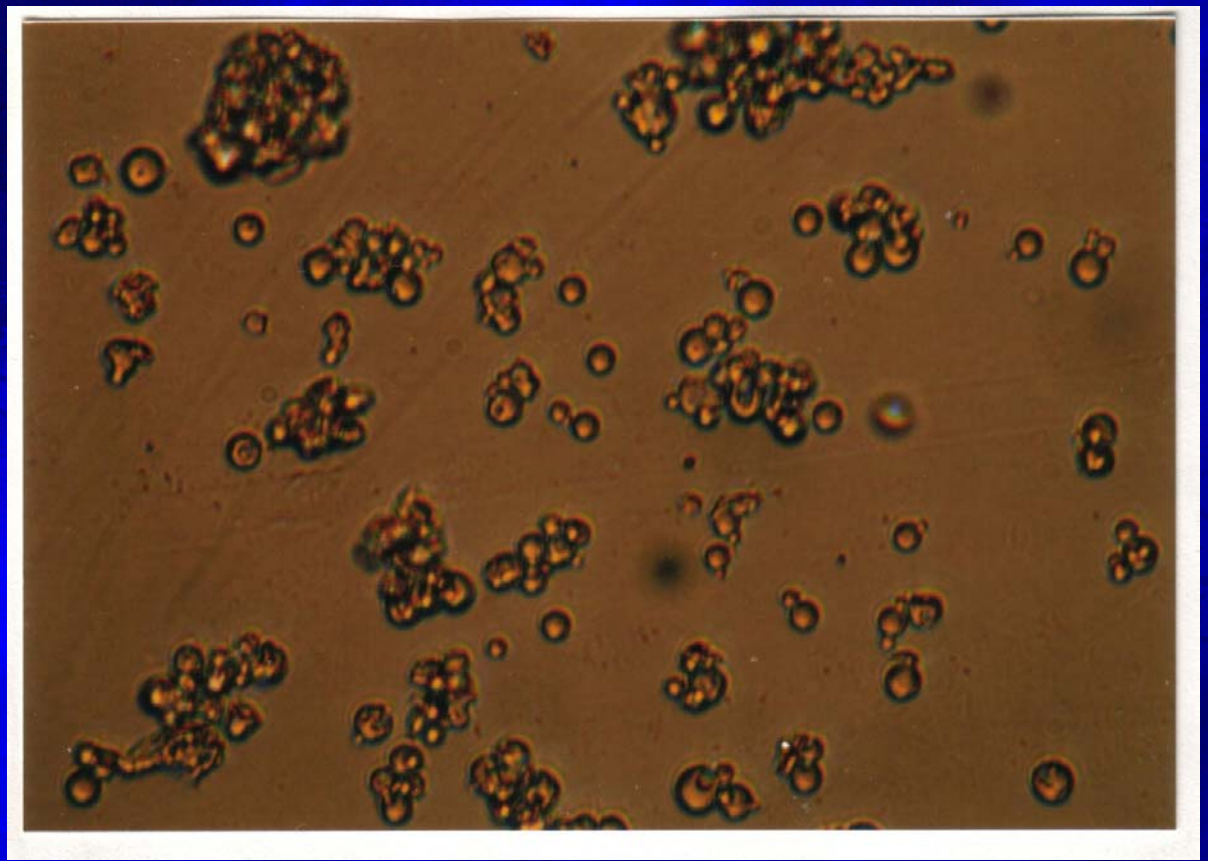
FORMULATION OF ACCURATE – DOSE – DETACHABLE – STERILE INSERTS (ADDSerts)

- The formulated ADDSerts consisted of a backing layer mode of 2% PVA having 5 x 20 mm dimension, a handle film, made of 3% sodium alginate with 5 x 15 mm dimension and a drug containing (225µg) 2% sodium alginate film 5 x 4 mm dimension. All the three polymeric layers were prepared separately by moulding technique.
- The handle and the drug containing film were placed over the backing layer, keeping 1 mm distance between them.
- Glycerin was used to stick these layers on to the backing layer.
- 4 different batches of ADDSerts were prepared using different grades of PVA namely, 14000, 22000, 33000 and 125000 molecular weight grades.

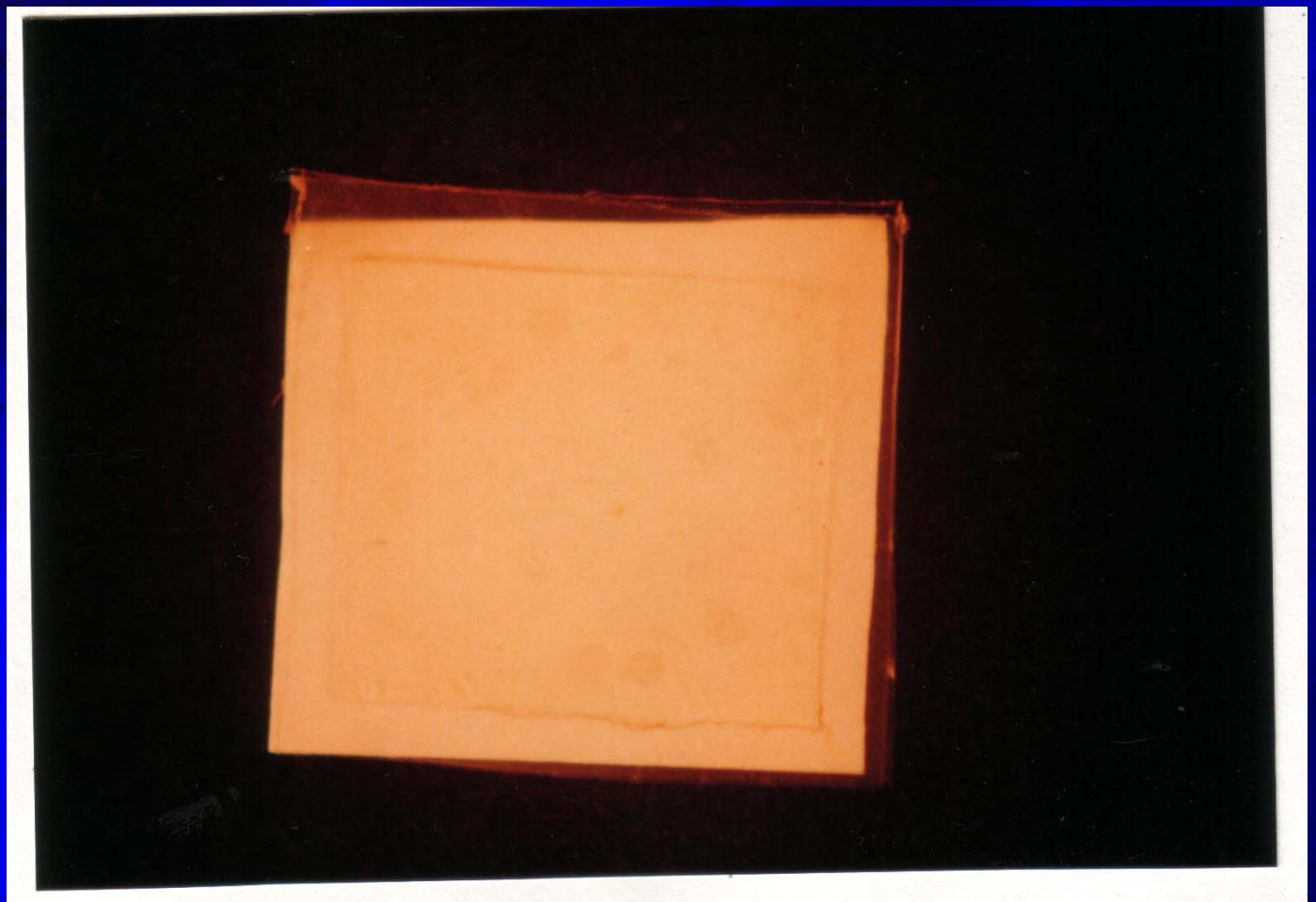
FORMULATION OF ACCURATE – DOSE – DETACHABLE – STERILE INSERTS (ADDSerts)

- An ideal batch was selected based on their detaching time and evaluated for its thickness, average weight, mucoadhesive strength, drug content and *in vitro* drug release
- Evaluation were carried out
- The detachment time was determined by immersing the formulated ADDSerts into the dissolution cell containing 0.5 ml of artificial tear fluid (37º C)
- The time for the detachment of the drug containing portion from the handle layer was determined

Physical appearance of ocular film



Physical appearance of MS-film



EVALUATION OF POLYMERS

- Effect of polymer concentration
- Effect of concentration of plasticizer
- Determination of film thickness
- Determination of average weight and weight variation
- Determination of mucoadhesive strength
- Determination of drug content
- In vitro drug release

STERILITY TESTING

- Moist heat sterilization of polymeric solutions for preparing Films, SC-Films and ADDSerts and

- Surface sterilization of microspheres for 2 min and aseptic dispersion in to sterilized polymeric solutions for preparing MS-Films were ideal for achieving sterility.

CORNEAL RESIDENCE

- Carried out in 6 numbers of albino rabbits, each weighing 2.5 kgs
- Rabbits were housed in individual cages and thoroughly examined for any ophthalmological abnormalities prior to use
- The study was planned in four phases in over night fasted rabbits
- Test delivery systems were applied to the lower conjunctival sac and their corneal residence, intraocular pressure (IOP) reducing potential and ocular safety were evaluated and compared with that of an equal dose of conventional drops (225 µg of drug)

STUDY DESIGN

Phase	Test Delivery system	Dimension & Dose applied
I	Ocular films	0.5 X 1 cm in size (=225µg TM)
II	Surface crosslinked ocular films	0.5 X 1 cm in size (=225µg TM)
III	Microspheres dispersed ocular films	0.5 X 1 cm in size (=225µg TM)
IV	ADDSerts	0.5 X 0.4 cm in size (=225µg TM)

HPTLC ANALYSIS OF TIMOLOL MALEATE

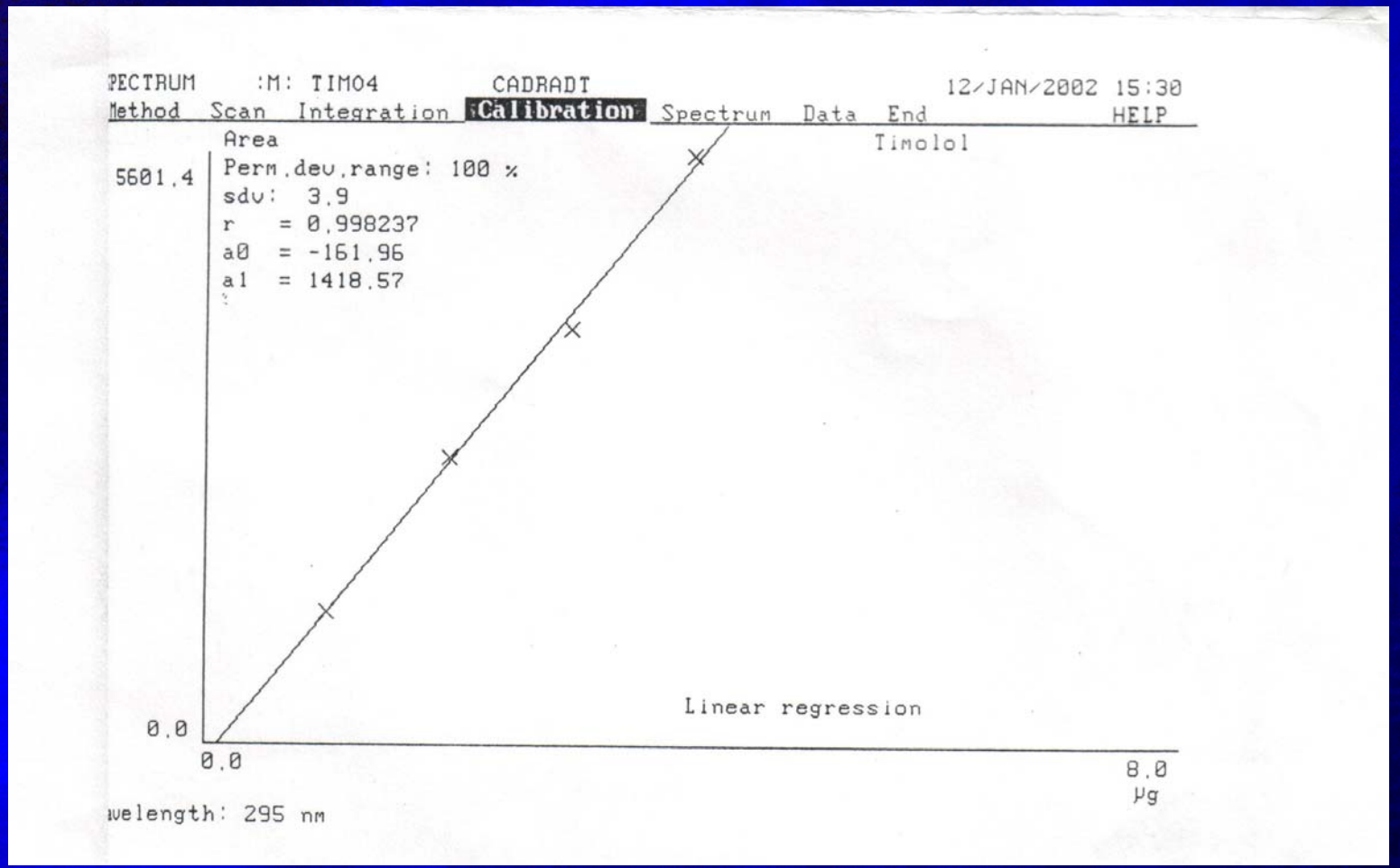
Materials

- CAMAG – HPTLC equipment
- Twin-trough development chamber (CAMAG)
- Linomat IV
- Stationary phase: precoated HPTLC Silica Gel GF plates
- Mobile phase: chloroform-methanol (8:2)

ANALYSIS BY HPTLC

- A Stock solution of timolol maleate equivalent to 1 mg/ml was prepared
- 1, 2, 3 and 4µl were spotted on to precoated Silica Gel GF plates (10 x 10 cm size)
- The plate was placed in the twin-trough development chamber, which was pre saturated with the mobile phase consisting of chloroform-methanol (8:2)
- The plate was developed
- Dried with a current of hot air
- Scanned at 295 nm in the densitometer
- Calibration curve was construted

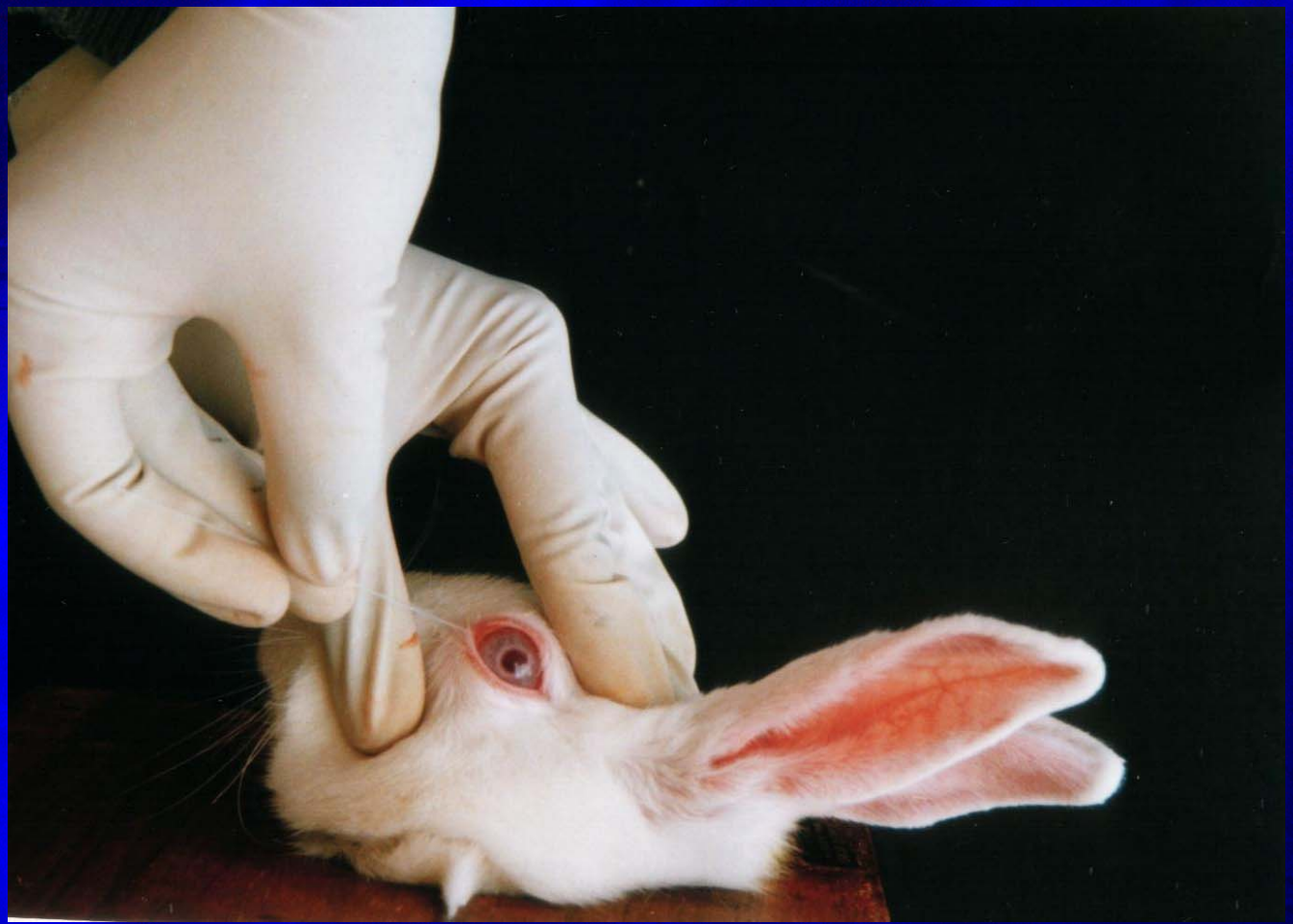
HPTLC Calibration curve of Timolol maleate



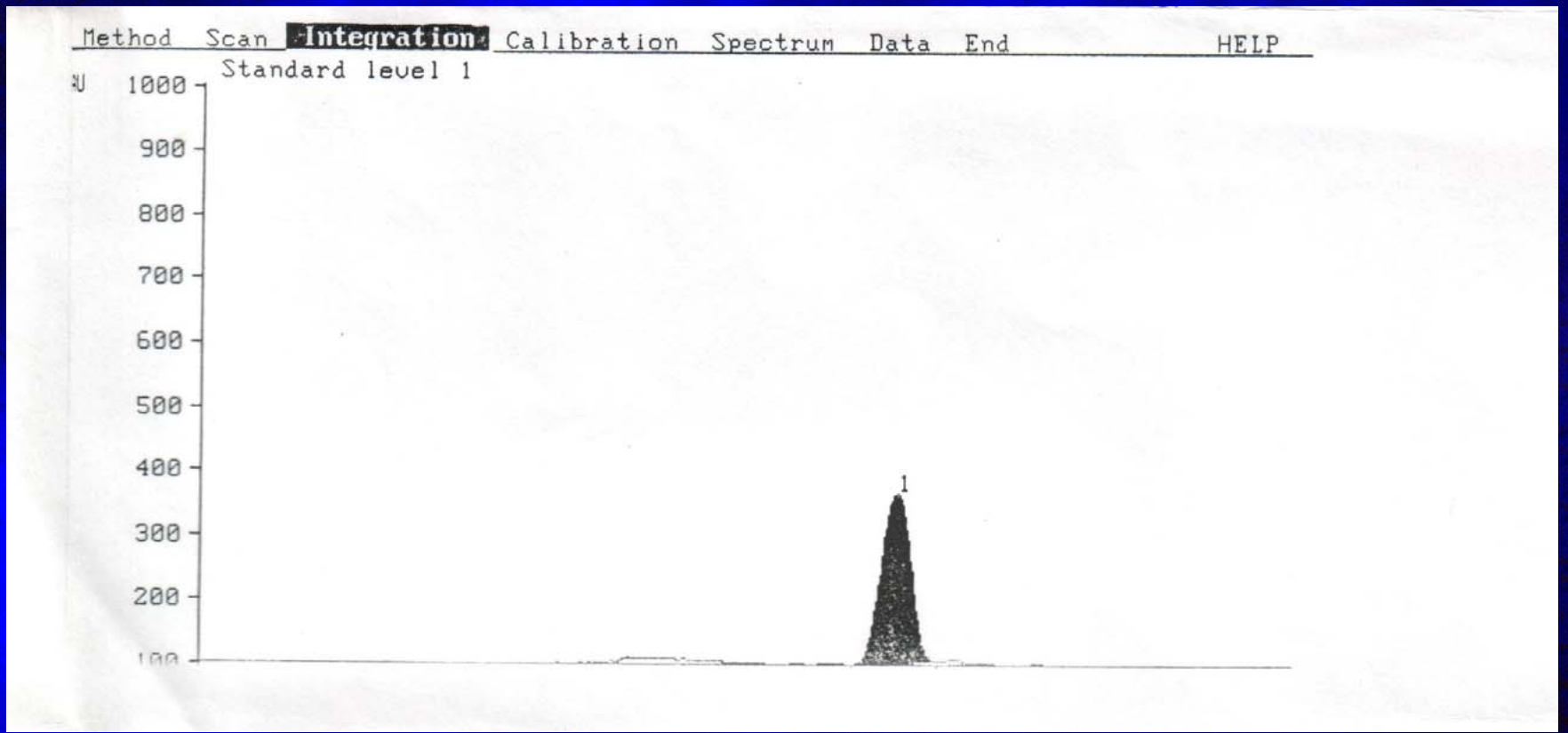
TEAR SAMPLING AND HPTLC ANALYSIS

- Tear samples equivalent to 1µl were collected from the left eye after application of the test delivery systems at 0, 0.5, 1, 2, 3, 4, 6, 8, 9, 12 and 24 h post dosing
- In case of conventional drops treated group, samples were withdrawn at 0.25 (15 sec), 5, 10, 15, 30, 45 and 60 minutes post dosing
- Glass Capillary tubes, having 320 µm internal diameter and 1 µl Premark were placed near the canthus of the eye with out applying pressure
- Tear fluid was drained in to the tubes due to capillary action

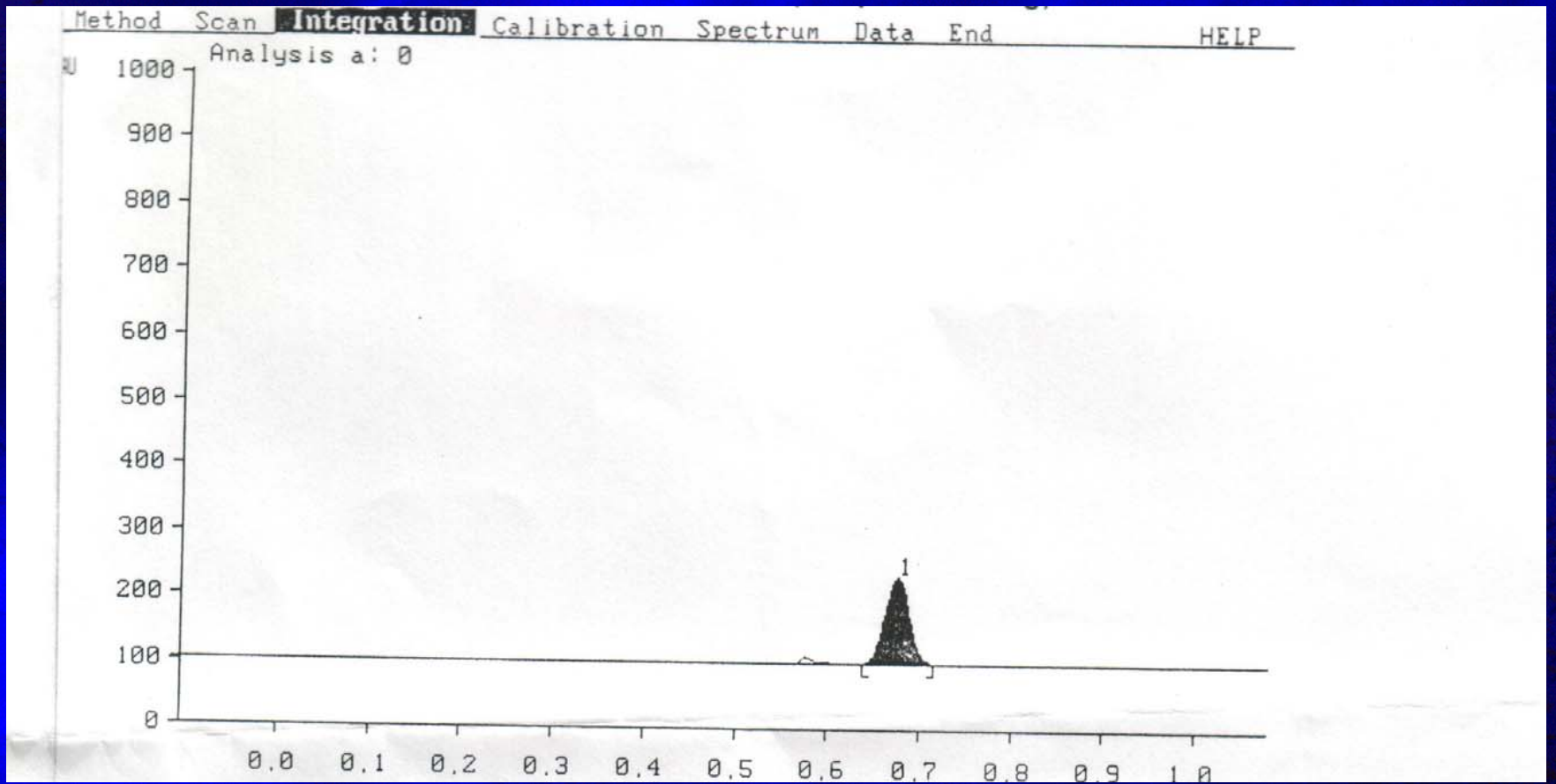
Tear fluid sampling



HPTLC Chromatogram of timolol maleate in tear fluid after convention drops application (5 mins post dosing)



HPTLC Chromatogram of timolol maleate in tear fluid after MS –Film application (9 hours post dosing)



RESULTS AND DISCUSSION

- Corneal Residence Evaluation : Conventional

  The drug levels at

  5 minutes -3.03 µg/µl

  10 minutes - 2.55 µg/µl

  15 minutes – 1.93 µg/µl

  30 minutes – No drug

Ocular films - Drug level maintained upto 8 h

- SC-film application : 12 h after - 0.63 µg/ml

TEAR FLUID CONCENTRATIONS OF TIMOLOL MALEATE

Treatment	Tear Fluid concentration (µg/µl) at different time (mins)						
	1	5	10	15	30	45	60
Conventional Drops	6.13	3.03	2.55	1.93	0	0	0

TEAR FLUID CONCENTRATIONS OF TIMOLOL MALEATE

Time (h)	Tear Fluid Concentration (µg)			
	Films	SC – Films	MS – Films	ADDSerts
0	0	0	0	0
0.5	1.51	0.76	0.80	1.28
1	2.40	1.64	1.83	2.20
2	3.10	2.20	2.80	3.00
3	3.00	2.73	3.42	3.11
4	4.00	2.71	4.41	3.98
6	5.62	2.10	3.85	5.46
8	1.8	2.20	2.80	2.30
9	0	2.00	2.80	0.82
12	0	1.5	2.00	0
24	0	0	0.63	0

TEAR FLUID CONCENTRATION – TIME CURVE

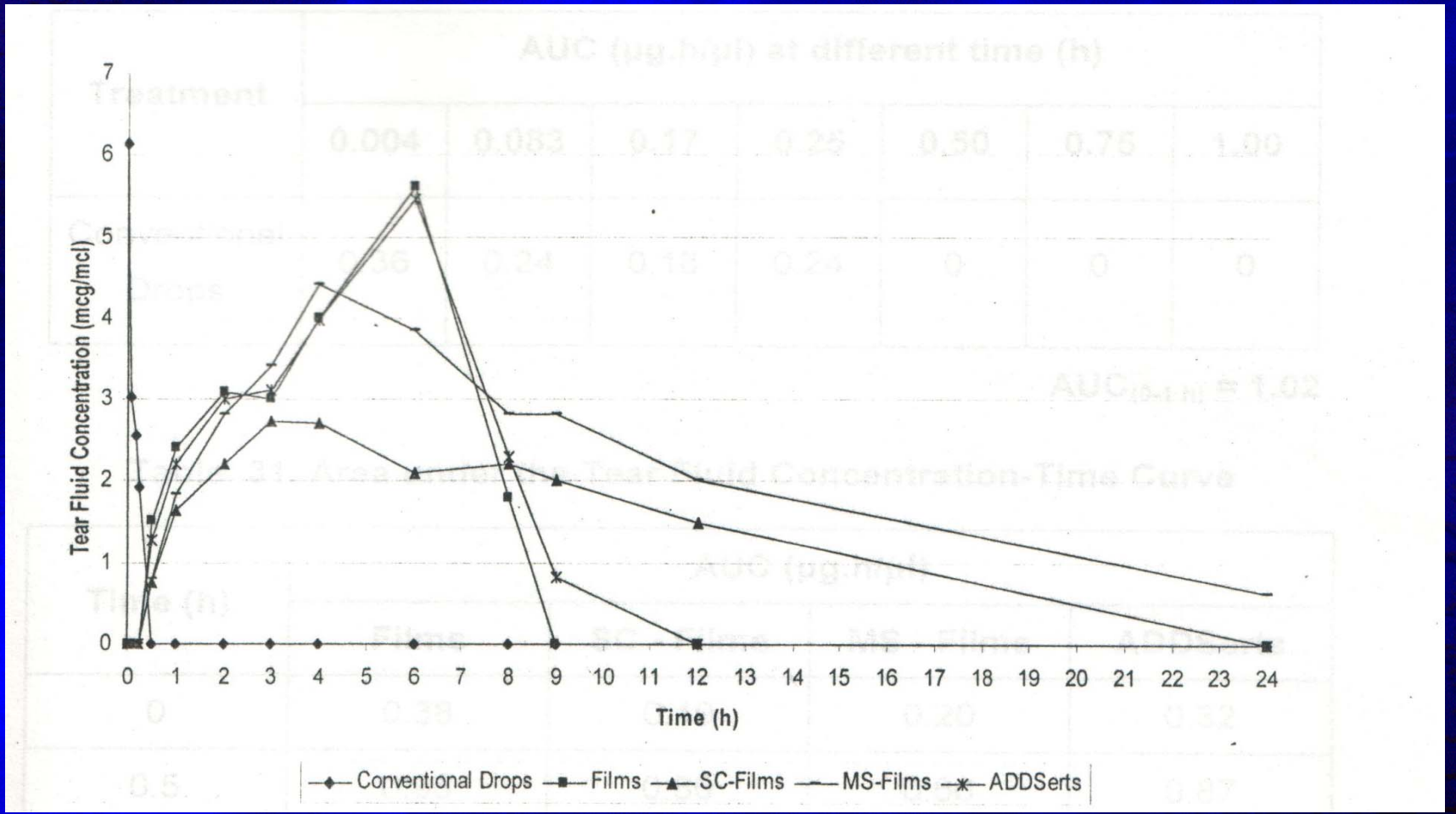
Treatment	Amount of Timolol maleate(µg/µl) at different time (minutes)						
	1	5	10	15	30	45	60
Conventional Drops	0.36	0.24	0.18	0.24	0	0	0

AUC (0-1 h) = 1.02

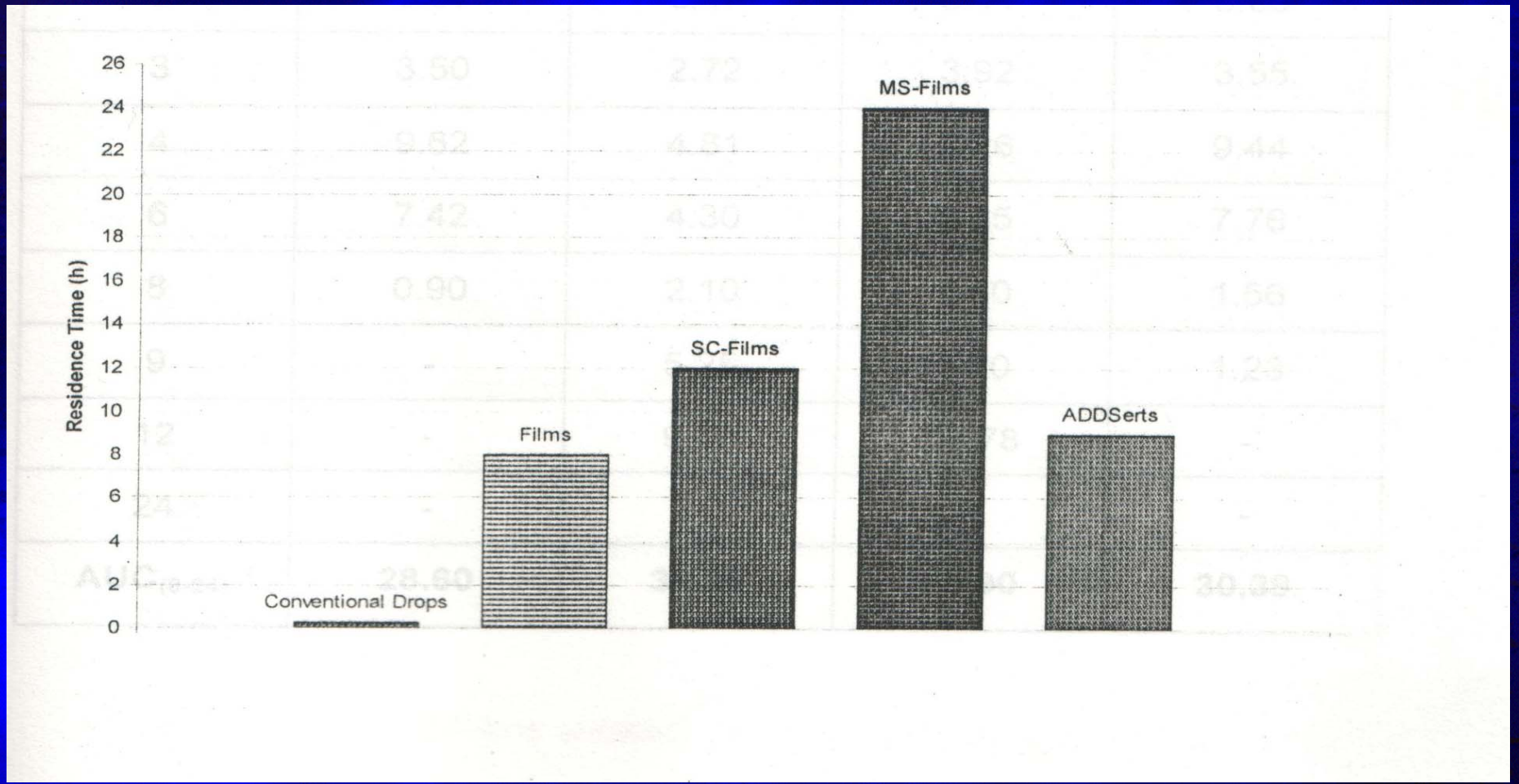
TEAR FLUID CONCENTRATION – TIME CURVE

Time (h)	Amount of Timolol maleate(µg/µl) at different time (h)			
	Films	SC – Films	MS – Films	ADDSerts
0	0.38	0.19	0.20	0.32
0.5	0.98	0.60	0.66	0.87
1	2.75	1.92	2.32	2.60
2	3.05	2.47	3.11	3.06
3	3.50	2.72	3.92	3.55
4	9.62	4.81	8.26	9.44
6	7.42	4.30	6.65	7.76
8	0.90	2.10	2.80	1.56
9	-	5.25	7.20	1.23
12	-	9.00	15.78	-
24	-	-	-	-
AUC (0-24)	28.60	33.36	50.90	30.39

Tear fluid concentration –time curve



Comparative precorneal residence




Thank You